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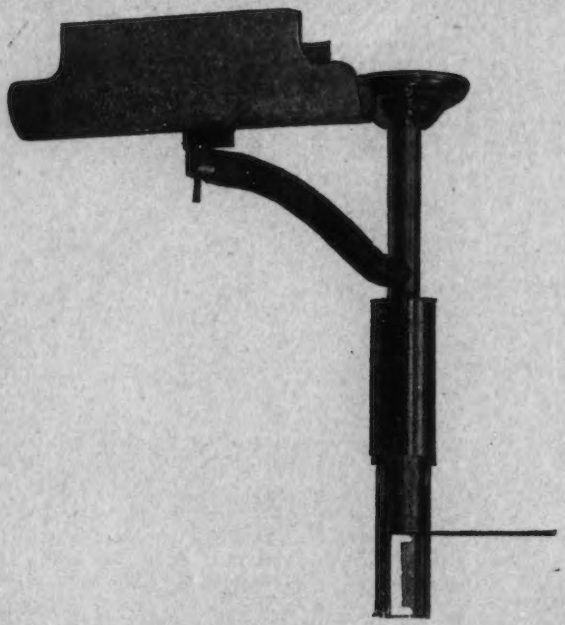

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THE DETERMINATION OF BLOOD CATALASE, WITH SOME OBSERVATIONS ON THE FACTORS AFFECTING THE RATIO BETWEEN QUANTITY AND ACTIVITY OF THIS ENZYME

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The work here reported was undertaken at the request of the Director of this laboratory in order to ascertain whether or not an adaptation of Van Thienen's (1) "Katalase Index" determination might be of value as a clinical method for distinguishing primary from secondary anemias. It was soon evident, however, that any attempt to make use of a catalase determination as an aid in diagnosis must be preceded by a very careful study of the reaction involved, and the factors bringing about differences in the results obtained. Because of the present tendency to attribute such large significance to catalase measurements in the interpretation of the mechanism of animal oxidations, as well as because of the supposed clinical possibilities of the reaction, it has seemed worth while to attempt to make such a study.

METHODS FOR THE DETERMINATION OF CATALASE ACTIVITY. Most American investigators have measured catalase activity, and thence, presumably, catalase concentration, by determination of the volume of oxygen liberated from hydrogen peroxide solutions by a given amount of the preparation containing catalase. It is obvious that such a method, if it is to claim reasonable accuracy, must take into consideration corrections for barometric pressure, temperature, tension of aqueous vapor, etc. Moreover, there must be an equalization of pressure in the

different parts of the apparatus before and after the generation of the gas, i.e., a system of leveling bulbs (2), and unless provision is made for the adequate and uniform shaking of the apparatus throughout the progress of the reaction, there will be errors due to the supersaturation of the reaction mixture with the oxygen liberated. Because of the complicated apparatus and careful manipulation required for a determination of this sort, its clinical application would be very limited.

It was considered preferable, therefore, to use for this study a method based on the determination of the hydrogen peroxide left in a solution after allowing the catalase preparation to act for a given length of time on a measured amount of hydrogen peroxide. An iodimetric method (3), which depended on the liberation of iodine from potassium iodide in acid solution by the hydrogen peroxide, followed by titration of the liberated iodine with thiosulfate, was first investigated. It proved unsatisfactory, however, because there was a tendency, after the action of the enzyme had been stopped by the addition of acid, for the oxygen held in solution to increase the amount of potassium iodide oxidized (4).

The method suggested by Senter (5) and used by Van Thienen (1) and other European investigators, proved to be more satisfactory. This consisted, essentially, in the estimation of the undecomposed hydrogen peroxide by titration of the acidified solution with standard potassium permanganate. The permanganate is reduced by the peroxide with the production of molecular oxygen, and the supersaturation of the solution with oxygen in no way interferes with the end point. The quantities of solution used by Van Thienen were so large as to be unwieldy. In order to maintain as nearly uniform conditions as possible throughout the progress of the reaction it seemed preferable, moreover, to make the separate titrations on determinations carried out in individual flasks, rather than to remove portions of the reacting mixture from time to time with a pipette. Hence the procedure adopted after a considerable amount of preliminary experimentation was as follows:

STANDARD METHOD: *a. Dilutions.* One cubic centimeter of whole blood[1] or corpuscles was measured with an Ostwald pipette into a 100 cc. volumetric flask partially filled with distilled water or 0.89 per cent sodium chloride solution.[2] The flask was filled to the mark with the

[1] Samples of human blood withdrawn from the arm vein were used throughout this investigation. Unless otherwise stated, these contained a small amount of powdered potassium oxalate.

[2] Unless otherwise stated, the enzyme solutions used in this investigation were distilled water dilutions of whole blood.

diluting fluid, the contents mixed, and further dilutions made in the same way, whole blood being diluted in the proportion of one part blood to 999 diluent for the standard catalase determination.

b. Determination of the activity of the enzyme. For the determination of the standard blood catalase value, 1 cc. of the neutralized 3 per cent solution[3] of hydrogen peroxide was measured with an Ostwald pipette into a very carefully cleaned and dried Pyrex Erlenmeyer flask of about 100 cc. capacity. This was diluted with 9 cc. of distilled water. Then 1 cc. of the enzyme solution described above was measured into the flask with an Ostwald pipette, and the time at which the enzyme was added carefully noted. The contents of the flask were then mixed by a rotary movement and the flask with its contents allowed to stand untouched for the desired length of time. When this had passed, the reaction was brought to an end by the addition of 10 cc. of 10 per cent sulfuric acid, and the contents of the flask titrated with a standard potassium permanganate solution of such a strength that 1 cc. of the solution[4] was equal to 2 mgm. of hydrogen peroxide. Control determinations were made in which 1 cc. of the hydrogen peroxide was diluted with water in the same way, 1 cc. of the boiled catalase solution was added, the contents of the flask allowed to stand for the same lengths of time, treated with acid, and finally titrated as described above. The figure for hydrogen peroxide decomposed was thus determined by difference.

It is possible to obtain very nearly exact checks on duplicate determinations by this method, if precautions are taken to eliminate errors due to imperfectly cleaned and dried apparatus, improperly calibrated burettes or pipettes, lack of uniformity in methods of delivering pipettes, etc., and proper care is exercised in timing the experiments. Each figure reported in this paper was obtained, unless otherwise stated, from duplicate titrations agreeing within 0.2 cc.

FACTORS AFFECTING THE RATIO BETWEEN THE AMOUNT AND ACTIVITY OF THE ENZYME. *A. Hydrogen ion concentrations.* The influence of hydrogen ion concentration on the amount of hydrogen peroxide decomposed by a given amount of catalase has been studied in detail by a large number of investigators. All of these agree that acid, or alkali in any

[3] Unless otherwise noted, the U. S. P. preparation put out by the Mallinckrodt Company, neutralized to a hydrogen ion concentration of pH 7.5 approximately, with 0.1 N NaOH was used.

[4] This requires, theoretically, 3.7195 grams of pure potassium permanganate per liter. The solutions used in this investigation were standardized by titration against recently standardized thiosulfate according to the usual iodimetric procedure.

considerable amount, destroys the enzyme, and that the optimum hydrogen ion concentration for its activity lies between pH 7.0 and pH 8.3. Practically all commercial hydrogen peroxides are acidified, since this substance decomposes more readily in neutral or alkaline than in acid solution. Michaelis and Peckstein (6) in their study of liver catalase, determined the effect of a wide range of hydrogen ion concentrations on the reaction. More recently the effect of variations in the hydrogen ion concentration of the reacting mixture on the quantities of hydrogen peroxide decomposed by the same amount of enzyme has been exhaustively summarized by Morgulis (7) and by Bodansky (8), whose results accord so well with those obtained in the short investigation included in the present study that it seems unnecessary to devote further space to a consideration of this factor. For all the determinations reported, the hydrogen ion concentration of the peroxide was adjusted to approximatly pH 7.5 by neutralization with 0.1N NaOH with the aid of outside indicators. It should perhaps be added that with human blood dilutions as a source of the enzyme, this insures a neutral reacting mixture.

B. Temperature. Much has been written concerning the velocity curves of the catalase reaction, and many of the statements in the literature seem contradictory. Careful examination of published reports reveals one thing, namely, that as a rule each investigator worked at one fairly constant temperature and assumed that the velocity constants which he calculated for that temperature ought to apply to a greater or lesser degree when the reaction was carried out under other conditions. To review the many papers which state that the reaction takes place according to the monomolecular law, or give reasons why it does not follow that law does not come within the scope of this paper. It is necessary, however, to consider what part variations in temperature play in affecting the amounts of hydrogen peroxide decomposed by given quantities of the enzyme in given periods of time. This can hardly be done without some references to the types of changes produced in the velocity curves by changes in reaction temperature.

Evans (9), working at a temperature of about 10°C., concluded that the curve of the reaction velocity went through a linear period in which the amounts of substrate converted in equal intervals of time were approximately equal, then through a logarithmic period, and finally through what he characterizes as "the period of marked slackening." This investigator apparently appreciated the fact that, other factors being constant, the linear period was greatly shortened at higher tempera-

tures; but he explained his results solely on the basis of relative concentrations of enzyme and substrate. And, curiously enough, the investigators who have used catalase determinations for clinical purposes, or for evidence as to the mechanism of animal oxidations, seem to have ignored almost completely the possible effect of change in the temperature at which measurements were made on their results.

It was apparent at the beginning of this investigation that the catalase values which were being obtained were distinctly lower than those reported for ordinary blood samples by Van Thienen (1) and by Nissen (10). After a number of possible causes for this discrepancy had been eliminated, it was remembered that the continental European room temperatures might be expected to be within the range 15 to 20°C., while in this laboratory the usual temperature range is about 24 to 26°C. Facilities for maintaining constant temperatures within the range 15 to 20°C. were lacking, but measurements on the same blood dilutions, all other factors being held constant, were obtained when the reaction was allowed to take place in a brine-cooled refrigerator at 4 to 6°C., in a cold room at 14 to 15°C., at the usual toom temperature[5] of about 25°C., and in an incubator bath at 35 to 37°C. These were made according to the standard method described above; the flask plus the hydrogen peroxide dilution and the enzyme solution were allowed to acquire the desired temperature before mixing, and the reacting mixture held at that temperature until the allotted time had elapsed, when the reaction was brought to an end by the addition of acid in the usual manner.

Such measurements were made with a large number of bloods of widely varying catalase content, and in each case a change in the temperature at which the reaction was allowed to take place affected the type of curve obtained in the same way. The set of curves shown in figure 1 has been chosen for publication, both because it is quite typical for a normal or approximately normal blood, and because this particular blood was used for a series of measurements of the effect of dilution of the enzyme which are to be given in table 1.

That the enzyme is increasingly destroyed as the temperature at which the reaction is allowed to proceed is set at a higher level, seems evident on inspection of these curves. Moreover, this conclusion is

[5] The heating of this laboratory is thermostat controlled. No experiments are reported in which the variation during the progress of the reaction was greater than 1°C., although one series (table 4) is given in which, because of an improperly adjusted valve, that variation lay between 28° and 29°C.

borne out by the fact that, once the reaction has reached equilibrium, neither addition of further amounts of hydrogen peroxide nor dilution of the reacting mixture with distilled water affects the amount of hydrogen peroxide decomposed.

What the mechanism of this destruction is, is a more difficult question to answer. Morgulis (11) has recently reported briefly experiments which indicate that the catalase reaction is exothermic. He concludes "the abnormalities of the reaction velocity are directly associated with the thermal phenomena which accompany the catalase reaction." However this may be, it seems quite reasonable to think that the enzyme may be destroyed progressively by the oxidizing action of the, for the instant, nascent oxygen which it liberates, and that both the amount of oxygen liberated and the amount of enzyme destroyed in the first few minutes of the reaction time are vastly increased at higher temperatures. It is apparent from the curves shown that the enzyme itself becomes more active as the level of temperature of the reacting mixture is raised, at least up to a certain optimum point. The amount of hydrogen peroxide decomposed in a given time is thus dependent on the relative activity of two forces, both of which vary with temperature.

The practical bearing of variations such as those shown by the curves of figure 1 on the significance of catalase values based on the amount of hydrogen peroxide decomposed or oxygen liberated by a given amount of a tissue preparation within a given time is very striking. On the basis of decomposition at the equilibrium point it is evident that we may obtain, from the same quantities of enzyme and peroxide, apparent catalase values which will diminish progressively as the level of the temperature at which the reaction is allowed to take place is raised. If the values are based on short time measurements, the variation is likely to be even greater and much more irregular. The time necessary to reach the equilibrium point becomes shorter as this temperature level is raised, and it is only at very low temperatures that the action of this enzyme resembles that of the typical inorganic catalyst. Moreover, the slight variations in room temperature which may occur when the measurements are carried out at different times of the day, or on successive days, is a factor which cannot be disregarded in interpreting the significance of comparatively small changes in catalase values. It is necessary, therefore, to choose and maintain a constant temperature throughout a series of measurements. Moreover, as the curves shown in figure 1 and the figures of table 1 indicate, if the stress is to be laid on the value obtained at the final equilibrium point, it is almost necessary to choose a temperature at or above 20°C., for purposes of comparison.

C. Concentration of enzyme. The conception of the relationship existing between concentration of enzyme and amount of substrate decomposed which one obtains from a cursory inspection of the literature is strangely confused. Most workers who have considered catalase determinations from the standpoint of physiological or clinical significance have merely assumed that the amount of hydrogen peroxide

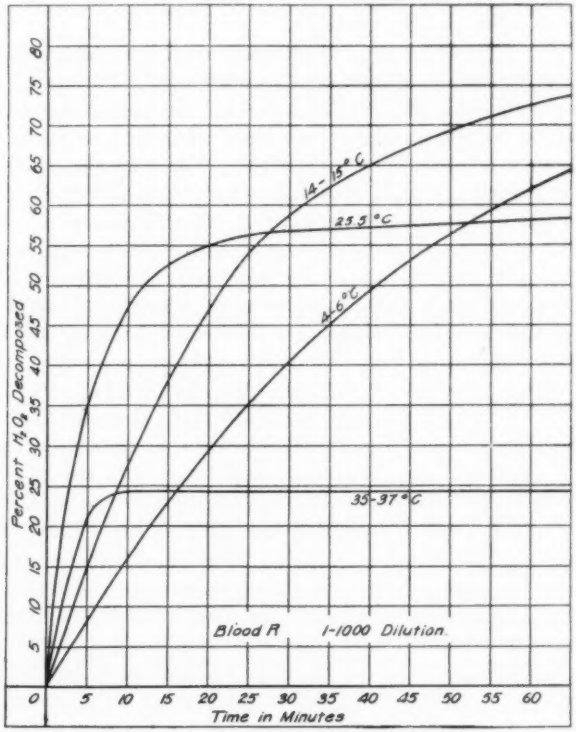

Fig. 1. Effect of temperature

decomposed in a given time or at the equilibrium point was directly proportional to the amount of catalase present in their enzyme preparation. On the other hand, the investigators who have approached the question from a physico-chemical viewpoint have worked out complicated, and quite frequently, different, mathematical expressions for the relationship existing between quantity of enzyme acting and reaction

velocity. Some of the general conclusions drawn are indicated very briefly below.

The earlier investigators of the nature of the reaction, notably Senter (5) worked with extremely dilute water solutions of the enzyme, and correspondingly dilute solutions of the peroxide. When the reaction was allowed to go on at 0°C.,the constants obtained were very nearly those demanded by the monomolecular law. Hence the conclusion that the enzyme acted as a true catalyst.

Evans (9), after an extensive investigation of the action of the enzyme carried out largely at temperatures under 10°C., considers that "No single law expresses the relation between concentration and activity of the enzyme solutions. Above a certain point increase in the strength of the catalase solution is without effect. For some distance from this point, the Schütz law (i.e., that the activity of the enzyme is proportional to the square root of its concentration) is followed. Increased dilution of the ferment gives a linear relation between the strength of enzyme solution and the amount of catalysis it can induce. With very great dilutions there is a rapid and irregular departure from this rule."

Morgulis (7) whose experiments were carried out at 20 to 21°C., states, "there is a direct proportionality between the amount of catalase and the amount of oxygen it produces from a given quantity of hydrogen peroxide. This proportionality holds only for the absolute quantity of catalase, changes in concentration having no direct effect on the amount of oxygen set free."

The present investigation was intended primarily to determine the extent to which, when the standard method described above was used, within the ranges met with in normal to markedly anemic bloods, the amount of hydrogen peroxide decomposed was actually proportional to the amount of catalase present. For this part of the experimental work, therefore, distilled water dilutions were made from bloods containing about 90 per cent hemoglobin (Palmer scale) and 40 per cent corpuscles (by volume). The results of typical measurements made according to the method already described are given in table 1. The curves showing the action of the 1–1000 dilution of this blood are charted in figure 1. These figures represent results where amount of enzyme only is varied, both concentration and amount of hydrogen peroxide being held constant, as well as the total volume of the reacting mixture.

It will be seen that, with 1–1000 dilutions of blood containing catalase in amounts varying from about one-fourth the normal to almost one-

third more than is normally present, the quantity of hydrogen peroxide decomposed *at the equilibrium point* may be expected to be directly proportional to the amount of enzyme acting, *provided the temperature at which the reaction is allowed to proceed is high enough.* When the concentration of the enzyme is very low or very high, there is a definite departure from this rule. Moreover, as the temperature is lowered, both the rate of decomposition of the peroxide and the rate of destruction of

TABLE 1

Relation between amount and activity of enzyme

BLOOD DILUTION	REACTION TEMPERATURE	CONTROL H_2O_2	HYDROGEN PEROXIDE DECOMPOSED											
			5 minutes		10 minutes		20 minutes		30 minutes		1 hour		∞	
			Milligrams	Per cent	Milligrams	Per cent	Milligrams	Per cent	Milligrams	Per cent	Milligrams	Per cent	Milligrams	Per cent
	°C.	mgm.												
1–2,000	4–6	25.6	0.6	2.3	1.8	7.1	3.8	14.9	5.8	22.7	11.1	43.5	16.7	65.5
1–1,000	4–6	25.6	2.6	10.1	4.0	15.6	7.4	28.9	10.6	41.4	16.4	64.1	25.6	100.0
1–4,000	14–15	26.0	1.2	4.6	1.6	6.1	2.4	9.2	4.0	15.4	5.2	20.0	6.0	23.1
1–2,000	14–15	26.0	2.6	10.0	4.0	15.4	6.0	23.1	7.6	29.2	9.6	36.9	10.6	40.8
1–1,000	14–15	26.0	4.0	15.4	7.4	28.4	12.4	47.7	14.4	55.4	19.0	73.1	25.6	98.4
1–8,000	25.5	25.6	0.8	3.1	0.8	3.1	0.8	3.1	0.8	3.1	0.8	3.1	0.8	3.1
1–6,000	25.5	25.6	1.2	4.7	1.5	5.8	2.0	7.8	2.0	7.8	2.0	7.8	2.0	7.8
1–4,000	25.5	25.6	2.8	10.9	3.2	12.5	3.6	14.0	3.8	14.8	3.8	14.8	3.8	14.8
1–2,000	25.5	26.2	4.2	16.0	6.2	23.6	7.2	27.5	7.6	29.7	7.6	29.1	7.6	29.1
1–1,000	25.5	26.2	9.4	35.9	12.4	47.3	14.4	54.9	14.8	56.7	15.2	58.2	15.2	58.2
1–666	25.5	26.0	12.6	48.4	16.6	63.9	19.6	75.4	21.0	80.8	21.6	83.1	22.4	86.1
1–500	25.5	26.0	16.0	61.5	19.6	75.4	23.0	88.5	24.4	93.8	24.8	95.5	25.6	98.5
1–2,000	35–37	26.0	3.0	11.5	3.2	12.3	3.2	12.3	3.2	12.3	3.2	12.3	3.2	12.3
1–1,000	35–37	26.0	5.4	20.8	6.4	24.6	6.4	24.6	6.4	24.6	6.4	24.6	6.4	24.6

the enzyme are decreased, and with unequal velocity. Hence, the relationship existing between the amounts of hydrogen peroxide decomposed in a given time, and the amount of enzyme acting, at temperatures lower than about 17 to 20°C., cannot be expressed by any simple proportionality.

Generally speaking, the lower the concentration of the enzyme, the shorter the time necessary to reach the equilibrium point. It is apparent from the table that, before the end point is reached, the

quantities of hydrogen peroxide decomposed in a given time may not be relied upon to be strictly proportional to the quantity of enzyme acting. While the experimental evidence on this point is limited to that obtained by the standard method described above, this suggests that there may be very valid reason for criticism of comparisons based on catalase values obtained from measurements made in short periods of time, e.g., 10 minutes, unless the relative concentration of enzyme in proportion to peroxide is extremely low. Moreover, it will be seen from the table, and it has been shown by numerous other experiments that, when the equilibrium point is reached in less than 20 to 25 minutes at our rather high room temperatures, the amount of enzyme acting is too small for the amount of hydrogen peroxide decomposed to be proportional to the catalase present. Since, at lower temperatures, the time required to reach the equilibrium point is increased, this fact may well be of considerable additional importance in the interpretation of the significance of the many catalase values in the literature which are based on the measurement of oxygen evolved in 10-minute periods. It should perhaps be stated that these conclusions have been borne out by a much larger series of measurements on normal and pathological bloods, to report which in detail would be a practical repetition which space does not permit.

D. Relative quantity and concentration of substrate. The literature dealing with the effect of variations in quantity and concentration of substrate on the catalase reaction is somewhat difficult of interpretation, because one type of investigator has based his conclusions on amounts of hydrogen peroxide decomposed or oxygen produced within a given short period of time, another on the amounts decomposed at the final end point, and a third on the rate at which the reaction proceeds.

Evans (9) states that for each concentration of enzyme there is an optimum concentration of substrate, and that these optima do not vary directly as the concentrations of the catalase, but more nearly as their square roots.

Morgulis (7) regards the reaction as reversible. He states that the maximum oxygen evolution occurs when 60 to 70 per cent of the total hydrogen peroxide is decomposed, and that when this maximum evolution of oxygen has been reached further increases in the quantity of hydrogen peroxide cause a falling off in the amount of oxygen set free by a given quantity of enzyme. When the concentration of hydrogen peroxide is increased without actual increase in the amount of peroxide in the reacting mixture, this investigator also reports depression of the

reaction, as he does when the amount of hydrogen peroxide is increased and a constant concentration maintained.

Again, the present study dealt primarily with the question of the effect of varying concentration and amount of hydrogen peroxide on the quantity of this substrate decomposed by blood catalase in such dilutions as one might expect to encounter in making measurements by the method outlined above.

The figures given in table 2 show the effect of varying concentration of peroxide, the amount being held constant, as well as the quantity and concentration of the enzyme acting. It will be seen that the higher concentrations of peroxide exert a depressing effect on the action of the enzyme in the early stages of the reaction, but that the equilibrium

TABLE 2

Variation in concentration of peroxide, total amount being constant

TOTAL VOLUME	CONCENTRATION H_2O_2	CONTROL H_2O_2	REACTION TEMPERATURE	HYDROGEN PEROXIDE DECOMPOSED											
				5 minutes		10 minutes		20 minutes		30 minutes		1 hour		∞	
				Milligrams	Per cent	Milligrams	Per cent	Milligrams	Per cent	Milligrams	Per cent	Milligrams	Per cent	Milligrams	Per cent
cc.	*per cent*	*mgm.*	*°C.*												
2.0	2.60	26.0	25.5	4.4	16.9	7.8	30.0	10.0	38.5	11.4	43.8	11.4	43.8	11.4	43.8
6.0	0.52	26.0	25.5	4.6	17.7	9.0	34.6	11.2	43.1	11.4	43.8	11.5	44.2	11.4	43.8
11.0	0.26	26.0	25.5	6.2	23.8	9.0	34.6	10.8	41.5	11.0	42.3	11.4	43.8	11.4	43.8
21.0	0.13	26.0	25.5	5.8	22.3	8.0	30.8	9.4	36.1	11.2	43.1	11.4	43.8	11.4	43.8

point reached is, within the limits of experimental error, the same regardless of concentration. The variations in concentration of peroxide used are not so great as those reported by Morgulis (7), but they do cover the range which will be encountered when commercial peroxides are used.

A discussion of the theoretical significance of these figures does not come within the scope of this study. It is hardly necessary to point out the further indication for making comparisons of catalase content on the basis of end point of the reaction, rather than on the amount of peroxide decomposed in a short period of time. The dilution represented by 0.26 to 0.32 per cent actual peroxide, i.e., 1 cc. of a commercial 3 per cent preparation plus 9 cc. of distilled water, was adopted for the standard method, largely because it gave a very convenient

total volume to the reacting mixture, and made it very easy to secure rapid and uniform mixing of the contents of the flask in which the reaction was carried out.

When the total amount of hydrogen peroxide is varied, the quantity and dilution of the enzyme being held constant, it will be seen by inspection of table 3 that there is a definite variation in the amount of hydrogen peroxide[6] decomposed. As Morgulis (7) observed, the optimum concentration is probably very nearly that which obtains when 65 to 70 per cent of the peroxide is decomposed. It is evident from the data given in table 3, and from many other experiments which cannot be reported in detail, that there is a range, between 25 and 60 per cent, approximately, within which the amount of hydrogen peroxide decom-

TABLE 3

Variations in amount of hydrogen peroxide, total volume constant

PROPORTIONATE AMOUNT H_2O_2	TOTAL VOLUME	REACTION TEMPERATURE	CONTROL H_2O_2	HYDROGEN PEROXIDE DECOMPOSED											
				5 minutes		10 minutes		20 minutes		30 minutes		1 hour		∞	
				Milligrams	Per cent	Milligrams	Per cent	Milligrams	Per cent	Milligrams	Per cent	Milligrams	Per cent	Milligrams	Per cent
volume	*cc.*	°C.	*mgm.*												
0.50	11.0	23.0	16.4	9.6	58.5	12.8	78.0	15.4	93.9	16.0	97.6	16.4	100.0	16.4	100.0
0.75	11.0	23.0	25.2	12.4	49.2	16.0	63.5	18.4	73.0	19.0	75.4	18.8	74.6	18.8	74.6
1.00	11.0	23.0	32.6	13.4	41.1	17.0	52.1	18.4	56.4	18.4	56.4	18.4	56.4	18.4	56.4
2.00	11.0	23.0	65.6	14.4	22.0	17.2	26.2	17.8	27.1	18.2	27.8	18.2	27.8	18.2	27.8
3.00	11.0	23.0	114.6	7.2	6.3	10.4	9.1	12.8	11.2	12.8	11.2	12.8	11.2	12.8	11.2
4.00	11.0	23.0	129.6	7.2	5.6	11.2	8.6	11.2	8.6	11.2	8.6	11.2	8.6	11.2	8.6

posed may be relied upon to be nearly proportional to the amount of enzyme acting than where the amount of peroxide approaches more closely to the optimum. This proportionality of hydrogen peroxide to enzyme, moreover, is within the range obtained when the proper dilution of the ordinary normal or pathological blood acts upon 1 cc. of a commercial 3 per cent peroxide according to the standard method given.

[6] The determinations reported in tables 3 and 4 were made with dilutions of Merck's "Perhydrol" corresponding in strength approximately to the U. S. P. preparations. As has been previously noted, results are exactly the same, whether this or the more easily obtainable commercial preparations are used, provided the dilution and hydrogen ion concentration factors are controlled.

E. The state of suspension of the enzyme: a. Laking the blood. That blood catalase is an endocellular enzyme, and is found in the blood cells rather than in the plasma is soon evident when determinations of the catalase content of cells and plasma from the same samples of blood are attempted. Nissen (2) made determinations by Van Thienen's (1) method on the catalase content of bloods diluted with water, and of the same bloods diluted with physiological salt solution. He obtained, in each case, a higher value for the salt suspension of the blood cells than for the laked blood. In view of the fact that our first preliminary experiments in which the dilution of the blood was made with physiological saline seemed to point to the conclusion that catalase values for a series of bloods tend to vary in a less consistent manner when comparisons are made on the basis of salt suspensions rather than distilled water dilutions of the cells, the matter was investigated further.

Suspensions in physiological saline and dilutions with distilled water were made in identically the same way with the same bloods and at the same time. The measurements were carried out simultaneously. A typical set of results is to be observed in figure 2 (curves *a* and *c*). It will be seen that when the corpuscles are suspended in physiological salt solution, the release of the enzyme is delayed, and consequently the catalase values obtained within the first 20 minutes or so[7] are lower than those for the water dilution. However, the destruction of the enzyme seems also to be delayed, as is to be expected from the slower release of the oxygen. The result is, therefore, a curve which, for the first hour or so, resembles that of the water solution of the enzyme acting at a lower temperature, and a somewhat larger total decomposition of peroxide.

To test whether or not the sodium chloride itself had any influence on the reaction, the hydrogen peroxide dilutions were made up in such a way that the resulting mixtures of enzyme and peroxide contained pure sodium chloride in a concentration of about 0.89 per cent. When the water solution of the enzyme was allowed to act in this medium (curve *b*) the effect was a slowing down of the rate of action of the enzyme, with a resultant end point slightly lower than that obtained when the enzyme solution was allowed to act on the same amount of peroxide diluted with water. On the other hand, when the solution of peroxide in 0.89 per cent sodium chloride was treated with the sus-

[7] The points indicated in figure 2 represent the experimentally determined course of curves *c* and *d*, which were "smoothed out" in the process of copying for reproduction. The points on curves *a* and *b* coincide with the lines.

pension of the enzyme in physiological saline, the result (curve *d*), for the first hour, at least, was a curve somewhat resembling those obtained when the experiments were carried out at a very low temperature. Unfortunately it is impossible, without distortion of the significant part of these curves, to show the whole course of the reaction in this

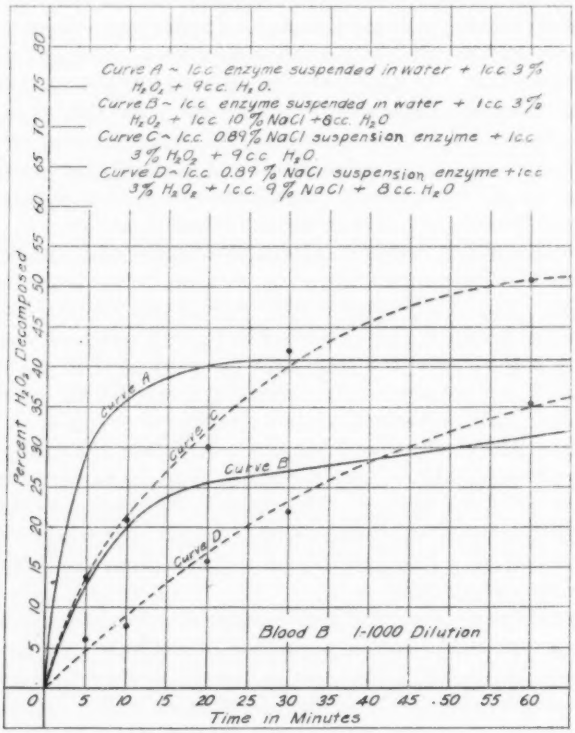
Curve A ~ 1 c.c. enzyme suspended in water + 1 c.c. 3% H_2O_2 + 9 c.c. H_2O.
Curve B ~ 1 c.c. enzyme suspended in water + 1 c.c. 3% H_2O_2 + 1 c.c. 10% NaCl + 8 c.c. H_2O
Curve C ~ 1 c.c. 0.89% NaCl suspension enzyme + 1 c.c. 3% H_2O_2 + 9 c.c. H_2O
Curve D ~ 1 c.c. 0.89% NaCl suspension enzyme + 1 c.c. 3% H_2O_2 + 1 c.c. 9% NaCl + 8 c.c. H_2O
Curve A
Curve C
Curve B
Curve D
Blood B 1-1000 Dilution
Percent H_2O_2 Decomposed
Time in Minutes
0 5 10 15 20 25 30 35 40 45 50 55 60
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80

Fig. 2. Effect of change in type of suspension of enzyme on reaction curve

manner. It must be noted, therefore, that these curves differ from those representing the course of the reaction at low temperatures in that the titration values obtained at intervals of 2, 6 and 20 hours after the beginning of the reaction show only a very slight increase in the amount of hydrogen peroxide decomposed over the values obtained for the one hour titration. Why this is the case can be explained only after

our knowledge of the mechanism of this reaction has been greatly augmented.

It has been shown repeatedly that the catalase of water dilutions of blood is not destroyed any more rapidly by standing after dilution than that of suspensions of corpuscles in normal saline. Hence it might seem that the change which takes place when the blood is laked represents rather an alteration in the state of dispersion of the enzyme than an actual destruction of the catalase.

The factor of reaction medium evidently affects very profoundly the type of the reaction curve. And it is manifest from inspection of a series of curves such as that given in figure 2 that the catalase values which might be taken from titrations at any point on the curves would differ according to the dilution medium used, as well as with the time when the corpuscles are laked. The values given by Van Thienen (1) and others who worked with Van Thienen's method seem to be based on the determination of the activity of physiological saline suspensions of blood, but the time allowed, i.e., 2 hours, was such that there is some possibility that they represent values near the equilibrium point, provided the temperatures at which the reactions were carried out were sufficiently high. The question of temperature control, together with that of irregularities in the reaction curve with the saline dilutions of blood, etc., is such, however, that this work should probably be checked up before it is accorded too much clinical significance.

The chief conclusion to be drawn from this phase of the study, practically speaking, is that, for measurements which are to be made within reasonably short periods of time, e.g., 30 minutes to 2 hours, water dilutions are to be preferred to saline dilutions of the enzyme.

b. Heating the enzyme dilutions. Of all the studies of the catalase reaction reported in the literature, those which raise the largest amount of doubt as to whether or not the catalase reaction may ever have clinical or physiological significance are probably the ones reported by Euler and his co-workers (12), (13), (14). Euler and Borgenstam (12) state that the heating of suspensions of the corpuscles of horse blood in physiological saline to temperatures of 55 to 57°C. increases the activity of the catalase to something like 170 per cent of the activity of the unheated enzyme. The full details of their measurements are not given, but the enzyme solutions were evidently heated in a double water bath with constant stirring, held at the desired temperature for half an hour, and cooled to the temperaure at which the catalase measurement was carried out. They explain this effect on the basis of increased "Dispersitäts-

grad," i.e., that the activation of the enzyme is due to its increased dispersion in the colloidal suspension. It is to be noted, also, that they made use of suspensions of the corpuscles from defibrinated horse blood as their source of the enzyme.

Efforts to repeat these experiments with the enzyme preparations that had been used for the rest of the measurements reported in the present study gave results which were neither entirely consistent nor satisfactory. In no case did heating of the water suspension of the enzyme produce more than a slight transitory increase in activity. In general, as is indicated by typical cases shown in divisions *1* and *2*, table 4, there seemed to be a gradual destruction of the enzyme. In one case, however, heating the physiological saline suspension of blood to 45°C. seemed to produce some activation of the enzyme. While this was so slight as to come almost within the limits of experimental error, it was deemed necessary to attempt to rule out the possible influence which might be attributed to the oxalate with which the blood samples had been taken, as well as the factor introduced by the presence of traces of serum proteins. Therefore, a sample of blood was taken without oxalate or citrate, defibrinated, and a suspension of the washed and centrifuged corpuscles made up in as nearly the fashion of Euler (12) as his description of his technique permitted. It should be noted, however, that human blood was used, and that it was withdrawn from the arm vein of a healthy individual under as nearly aseptic conditions as possible, while this investigator used horse blood and made no statement as to the conditions under which it was obtained. Moreover, he found that his increased activation was in direct proportion to the time during which he incubated his suspensions at 55 to 57°C. The results of measurements on the enzyme preparation last described, heated and unheated, are indicated in the third division of table 4. It will be seen that, at the end of 5-or 10-minute periods, Euler's conclusion as to increased activity might be supposed to hold good. But increasing the time during which the enzyme was allowed to act led to the conclusion that some of the catalase had either been killed or inactivated by the heating process. In the solutions which had been heated to 55°C., moreover, the corpuscles were almost completely laked, so that we may perhaps have reason for the assumption that the apparent increase in activity during the first few minutes of the reaction was due to the fact that the cell walls had been broken down, and that the enzyme was therefore more quickly available. It should be noted that the room temperature on the day that these experiments were carried

out was particularly high, and that the measurements were made immediately after the heated enzyme suspensions had been cooled to that temperature.

It is recognized that the discussion of the general question of the effect of alterations in the degree of dispersion of colloidal enzyme on its

TABLE 4

Effect of heating enzyme suspensions

SOURCE OF ENZYME	CONTROL H_2O_2	HYDROGEN PEROXIDE DECOMPOSED									
		5 minutes		10 minutes		20 minutes		30 minutes		1 hour	
		Milligrams	Per cent	Milligrams	Per cent	Milligrams	Per cent	Milligrams	Per cent	Milligrams	Per cent
	mgm.										
I. Oxalated whole blood,* water dilution											
a. Unheated	31.4	8.2	26.1	9.8	31.2	10.4	32.4	10.6	33.7	10.6	33.7
b. Heated to 45°C., cooled†	31.4	7.6	23.7	9.0	28.0	9.0	28.0	9.0	28.0	9.0	28.0
c. Heated to 55°C., cooled†	31.4	6.8	21.7	7.6	23.7	8.6	27.4	8.6	27.4	8.6	27.4
II. Oxalated whole blood,* 0.89 per cent NaCl dilution											
a. Unheated	32.4	4.2	13.0	5.6	17.3	10.8	33.3	12.2	37.7	13.6	42.0
b. Heated to 45°C., cooled†	32.4	4.2	13.0	6.2	18.7	10.2	31.5	13.0	41.0	14.0	43.2
c. Heated to 55°C., cooled†	31.2			7.8	25.0	10.6	33.9	12.2	39.1	12.2	39.1
III. Defibrinated blood, 0.89 per cent NaCl dilution											
a. Unheated	30.8	2.4	7.8	4.4	14.3	7.2	23.4	8.4	27.9	8.4	27.9
b. Heated to 55°C., cooled†	30.8	4.6	14.9	5.8	18.8	6.4	20.8	6.4	20.8	6.4	20.8

* Samples I and II were 1–1000 dilutions of the same blood.

† Cooled to room temperature, which was 28–29°C. when all these measurements were made. See text for details.

activity does not lie within the scope of this paper. Without going further, therefore, the writer merely wishes to say that the results of Euler in this case have at best been only very imperfectly confirmed. Irregularities in the measured activity of catalase preparations which have been heated are such, however, that there is every reason to

avoid exposure of bloods which are to be used for comparison of catalytic activity of temperatures higher than that at which the measurement is to be made.

F. Salts and antiseptics. The effect of the presence of these substances on the catalase reaction has been studied in great detail by Senter (15), Euler (12), (13), (14) and others. Senter concluded from his investigations that alkali and alkaline earth ions had no effect on the activity of the enzyme; that the chloride, bromide and iodide ions tended to destroy it, as did nitrates, chlorates and perchlorates, persulfates to a lesser degree. Hydrogen sulfide, iodine, mercuric chloride, and mercuric bromide, were, as one would expect, found to be very toxic, while mercuric cyanide has a much less marked effect. Carbon monoxide, arsenic trioxide and formaldehyde were considered to be inactive.

Euler, Blix and Laurin (13), (14) studied the activation of the catalase of yeast by the addition of small amounts of poisons. The discrepancies between their findings for yeast catalase and the findings of Euler and Borgenstam (12) for the catalase of horse blood arouses some doubt, however, as to the extent to which their conclusions may be expected to apply to human blood. They found, in the case of the yeast catalase, marked activation produced by the addition of small quantities of toluene, thymol, chloroform and "other narcotics which either do not combine with the cell contents, or combine with them very slowly."[8] Phenol, in the presence of a buffer solution, gave a marked effect of increased activity. With the catalase of horse blood, however, the addition of chloroform gave a much slighter increase in activity, and toluene had little or no effect.

The present study is concerned only with the effect of such substances as might be likely to be added to bloods as preservatives. Of these, perhaps the most commonly used are toluene, xylene, chloroform, formaldehyde and, of course, oxalate. Acetanilid is so usually present in commercial hydrogen peroxide solutions that its action is also of interest. The study of the effect of these substances was carried out in exactly the same way as the experiments already described in detail, but it seems best to give only a summary of the findings, since the measurements in themselves present nothing new.

Toluene and *xylene* have been shown repeatedly to have no effect on the activity of the enzyme, when added to the blood dilutions. *Chloroform* saturation of the diluted enzyme preparation increased its activity about 10 per cent, at the equilibrium point, which was reached at 26.5°C.

[8] The writer's translation.

in a little more than 20 minutes. *Oxalate* in strengths as great as would be represented by the addition of 1 gram of potassium oxalate to 1 cc. of blood, i.e., 1 mgm. to 1 cc. of the dilutions used, had little or no effect. The amounts of *acetanilid* which might be expected to be found in commercial preparations of hydrogen peroxide (the usual U. S. P. solution contains about $\frac{3}{16}$ grain per ounce) had practically no effect. In considerably greater concentrations, however, (5 mgm. per cc. of the hydrogen peroxide solution called for by the standard method outlined above) a decrease of about 10 per cent in the amount of hydrogen peroxide destroyed was obtained. *Formaldehyde* in the strength recommended to preserve blood filtrates for sugar determinations, i.e., 1 cc. of the 40 per cent formalin solution per 150 cc. of the blood dilution, had a very marked effect in decreasing the amount of peroxide destroyed.

Apparently, therefore, xylene and toluene are the best preservatives for blood dilutions to be used for catalase determinations. There seems to be no reason why the blood samples should not be taken with a moderate amount of oxalate; in fact, there is very good reason to think that blood samples taken in this way in sterile or nearly sterile containers are more reliable than samples which must inevitably become more or less contaminated in the process of defibrination. On the other hand, chloroform, formaldehyde, etc., are to be avoided.

SUMMARY. The details of a micro adaptation of the permanganate titration method for the determination of catalase in whole blood or corpuscles have been described.

The effect on the relationship between catalase concentration and catalase activity as manifested by decomposition of hydrogen peroxide, of temperature level, variations in relative amount and concentration of enzyme and substrate, and in the state of dispersion of the enzyme has been studied, as well as the effect of adding some common salts and preservatives.

CONCLUSIONS

The values obtained in the determination of the catalase of human blood vary greatly with variations in the conditions under which the reaction is allowed to proceed. It is necessary, therefore, in order to obtain figures which are comparable for interpretation in terms of clinical or physiological significancance, to control these conditions very carefully.

As the temperature at which the measurements are made is raised above 0°C., the curve of the decomposition of hydrogen peroxide by

catalase varies widely from that to be expected in a true catalytic decomposition. When the temperature is raised, equilibrium is reached in a shorter time, and the reaction velocity becomes greater, but, because of increased destruction of the enzyme, a smaller total amount of hydrogen peroxide is destroyed. Comparatively small day to day and hour to hour variations in room temperature may be sufficient to account for many of the smaller variations in catalase content of tissues reported in the literature. If such a value is to be based on a single determination of hydrogen peroxide decomposed, the reaction should be allowed to proceed at 20° C., or a slightly higher temperature, and at least one hour allowed for reaching the equilibrium point.

Within certain limits, i.e., when the amount of peroxide decomposed is about 25 to 60 per cent of the total, and the amount and concentration of hydrogen peroxide is kept constant throughout a series of measurements, the amount of peroxide decomposed at the equilibrium point may be relied upon to be directly proportional to the amount of enzyme acting, provided, of course, other reasons for variation are eliminated. Too great relative variation in the concentration of enzyme or substrate will lead to a departure from this relationship.

Water dilutions of blood or corpuscles give catalase values which are probably more nearly reliable for purposes of comparison than are those from suspensions of blood in physiological saline.

The activation of the enzyme reported by Euler and his co-workers to be produced by heating saline suspensions of blood corpuslces to 55 to 57°C. seems to be a transient effect apparent only during the first 10 minutes of the reaction, and possibly due to the laking of the corpuscles. Measurements of the activity of the heated dilutions continued for longer periods of time indicate that there is some inactivation of the enzyme, as do also measurements with heated water dilutions of the blood.

The use of certain preservatives, notably chloroform and formaldehyde, must be avoided in the preparation of solutions for determinations of catalase activity. The use of oxalate, toluene and xylene is permissible

The writer is not, at the present time, prepared to express an opinion as to the extent to which the determination of catalase activity may have clinical or physiological significance. It is apparent, however, that such determinations must be made under much more carefully controlled conditions than in the past, if they are to admit of interpretation for diagnostic purposes, or if variations in observed catalase content of blood,

tissues, etc., are to be taken to indicate the trend of oxidative processes in the animal organism.

The writer is indebted to Dr. R. B. Gibson, the director of this laboratory, for his suggestion in connection with this work, and to Dr. G. Ferreira of the medical staff for his very kind coöperation in securing the blood samples.

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STUDIES IN FATIGUE

XII. The Effect of Adrenal Secretion on Non-Fatigued and Fatigued Skeletal Muscle

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In 1913 Cannon and Nice (1) showed that, with adrenal glands intact, splanchnic stimulation markedly increased the height of muscular contraction in fatigued skeletal muscles. When all the adrenal vessels were ligated this betterment did not result and if the adrenal veins were clamped it was delayed until the clamps were removed. The following year Gruber (2) noted that fatigue increases the threshold of the nerve-muscle preparation. He also noted that splanchnic stimulation for from 30 seconds to 1 minute was capable of reducing this increased threshold from 23 to 94 per cent of its original height. The question of the action of sympathetic stimulation and adrenal secretion upon the contraction of non-fatigued skeletal muscle was not considered by these experimenters. Recently Gruber (3) showed that adrenalin injected intravenously had the same effect upon non-fatigued muscle as it had upon fatigued skeletal muscle. The present work was undertaken to see whether adrenalin when secreted has the same action as when injected intravenously in moderate or massive doses.

Method. Cats were used under ether anesthesia. The method was the same as that used in a previous communication (3) and only the part not described there will be reported here. The time interval in all cases was 30 seconds. The rate of stimulation was 35 stimuli per minute for the fatigued muscle and 3 stimuli separated by periods of 10 seconds followed by a rest of 1 minute for the non-fatigued muscle. Both muscles were after-loaded by 50 grams.

By a median abdominal incision the right adrenal gland was exposed, separated from the surrounding tissues and its blood vessels ligated. Care was taken to keep the organs thus exposed during the

operation warm and moist by covering them with a towel moistened with warm Ringer's. The left greater and lesser splanchnic nerves were isolated and ligated centrally and shielded electrodes similar to those used by Cannon and Nice (4) were applied distally to them. The abdomen was closed by fastening together the two edges of cut skin with paper clips. The splanchnic nerves were excited by a moderately strong, rapidly interrupted induction current. The duration of stimulation in each case was about 1 minute.

Results. Figure 1 is presented as showing the characteristic action of adrenal secretion upon non-fatigued and fatigued skeletal muscles. The left splanchnic nerves were excited at *1*, with the left adrenal gland intact; at *2*, after the adrenal vein was clamped; the clamps were removed at *b* and at *3*, after the blood supply to the left adrenal gland was again shut off.

As a result of the first stimulation at *1*, the fatigued muscle showed a maximum increase of 52 per cent in the height of contraction, the betterment lasting for 6½ minutes. This increase was 38 per cent higher than the initial height of contraction before fatigue. The non-fatigued nerve-muscle showed increased height of contraction lasting for 11½ minutes with a maximum increase in the height of 50 per cent. The mean blood pressure temporarily mounted from 125 to 140 mm. of mercury (15 mm. increase) which soon fell to 115 mm. (a drop of 25 mm.) remaining thus for some seconds.

The height of the contraction of the fatigued muscle increased, at *2*, 23 per cent but was beginning to decline when the clamp was removed from the adrenal vein at *b*. Immediately the height of muscular contraction was thus increased by 41 per cent or a total of 64 per cent which was 20 per cent higher than the original height of muscular contraction before fatigue.

The height of the contraction of the non-fatigued nerve-muscle increased 16 per cent when the adrenal vein was clamped and the splanchnic nerves stimulated but a further increase of 25 per cent was obtained when the clamp was removed from the adrenal vein (total 41 per cent). The blood pressure rose upon splanchnic stimulation from 105 to 120 mm. of mercury but soon fell to 100 mm. and gradually returned to normal.

Upon releasing the clamp on the adrenal vein the blood pressure again fell below the 105 mm. of mercury level at which point it remained for some seconds.

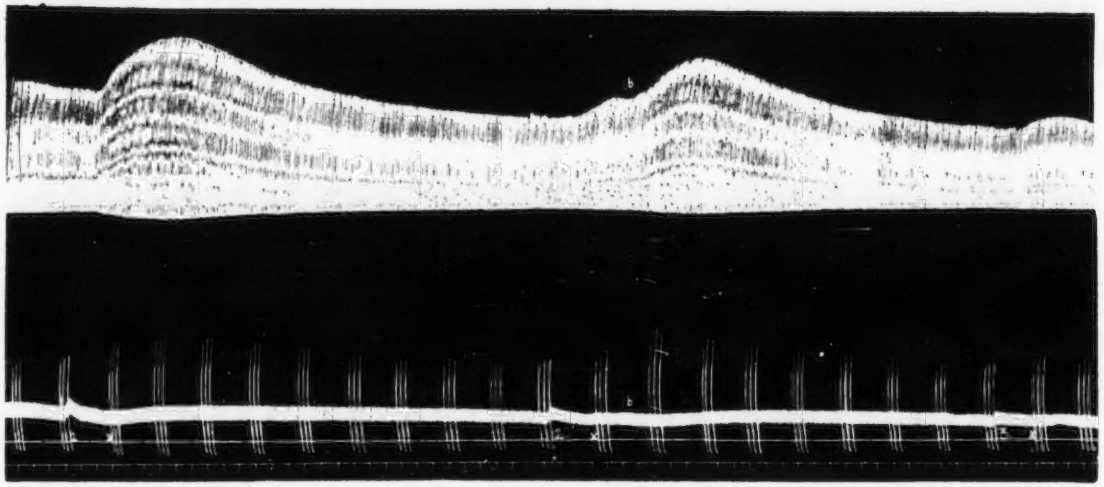

Fig. 1. Upper record, the right anterior tibial muscle stimulated indirectly 30 times a minute, lifting a load of 50 grams. Below it the blood pressure with a membrane manometer. Third record from the top, the non-fatigued muscle stimulated three times every 1½ minutes, the stimuli separated by intervals of 10 seconds. Lowest record, time in 30 seconds and zero blood pressure. *1* to *x*, stimulation of left greater and lesser splanchnic nerves with the left adrenal gland intact; *2* to *x*, stimulation same as in *1*, but the left adrenal vein was clamped to point *b*, when the clamps were removed; *3* to *x*, stimulation same as in *1*, but the left adrenal vein to left adrenal gland was again clamped. Curve reduced ⅓.

Splanchnic stimulation at *3* produced an increase of only 10 per cent in the height of contraction in both the non-fatigued and fatigued nerve-muscles. The blood pressure changed from 100 to 115 mm. of mercury. The betterment therefore produced by the change in blood pressure is very small. Gruber (5) showed that a large change in blood pressure is necessary to cause a noticeable change in muscular contraction when the original pressure is above 90 to 100 mm. of mercury which he termed the "critical region." The betterments produced in non-fatigued and fatigued nerve-muscles upon splanchnic stimulation are therefore mainly the action of the adrenalin secreted and carried by the blood to these excited tissues.

SUMMARY

1. Adrenal secretion evoked by splanchnic stimulation increases the height of muscular contraction of both the non-fatigued and fatigued skeletal muscle. The quantity of adrenalin secreted must be fairly large judging from the height of betterment and the duration of improvement when compared with the results from injections of adrenalin intravenously.

2. In as much as adrenalin secreted by splanchnic stimulation affects non-fatigued and fatigued skeletal muscles alike, it cannot be regarded a specific antagonist to the so-called fatigue substances. It increases the height of contraction and irritability of both muscles. Its exact mode of action has not been determined.

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THE OUTPUT OF SUGAR FROM THE LIVER AS AFFECTED BY C_H AND MINIMAL EPINEPHRIN

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The reverse reaction of minimal amounts of epinephrin having been demonstrated for the portal-venous system of the terrapin's liver by two of us (1), the question at once came up as to how the output of sugar by the liver under similar conditions is affected. Experiments accordingly were carried out to answer this question.

The terrapin's liver was again used and the method of perfusing it was identical with the method described in the former work. Ringer's buffered with phosphates was again used, only in some of the experiments a small amount of dextrose (Pfanstiehl) was added. Four reservoirs contained the solutions, two of which were set at lower and two at higher pH; to one of each of these pairs adrenalin-Takamine was added in the ratio of $1:10^9$. Instead of using the drop-recording apparatus and allowing the outflow from the hepatic veins to waste, it was collected in small graduated flasks (50 cc.) and the time required for filling to scratch mark observed on a stop-watch. The outflow could thus be reduced in all cases to definite time periods for comparison. In one case, experiment of January 26, the liver was entirely excised and the outflow from the lymph vessels as well as the hepatic veins collected.

It should be added that the livers were perfused for some time before samples were taken, allowing the organ thus to adjust itself to the fixed conditions of the experiment,—the saline perfusion fluid, its hydrostatic pressure, temperature, etc.—the exact data of which are recorded in the reports given below. Some time was required to free the liver of most of its blood; the first 50 to 100 cc. collected were accordingly discarded. But at all times protein continued to be carried out by the perfusates and appeared in all the samples. The complete removal of this was found to be best accomplished by dialyzed colloidal iron. The analyses for reducing substances

were carried out first by the method of Bertrand, but later entirely by the modification of Bang's method as described by Shaffer and Hartman (2). This latter method was found to be highly satisfactory. The samples were at first not always immediately analyzed at the time they were collected, boiling and covering with toluol being relied upon to preserve the reducing substances. The observation that these substances may be converted into non-reducing substances upon standing in the presence of blood corpuscles even though micro-organisms are absent (see Macleod, 3b, for literature and discussion of this) would not apply in our experiments. Nevertheless the analyses were finally all made immediately after the samples were collected. Briefly put, the procedure consisted of *a*, removal of protein; *b*, filtering; *c*, concentrating on the water-bath, exact original volumes always being noted to which the sugar content was finally referred; and *d*, analysis for reducing substances.

Tables 1 and 2 give the results of five experiments. Both give the minute-volume and the sugar output reduced to equal time intervals. Table 2 contains in addition the observed time required to collect a given volume of outflow, thus furnishing the data upon which the minute-volume calculation is based. Under the heading "character of solution" are indicated the C_H of each solution, in terms of pH, and the presence (plus sign) or absence (negative sign) of adrenalin. Finally is added the amount of reducing substances found in the sample analyzed (50 cc.) and adjusted for the amount that would be found in an outflow of exactly 10 minutes in each case. It was very early evident that the output of sugar from the liver into the vena cava depends very much upon the time. The importance of this method of comparing the output in preference, say, to actual concentration in the hepatic outflow will be taken up again in the discussion to follow. In the later experiments dextrose was added to the Ringer's solution to make the concentration about 0.05 per cent. This amount is corrected for in the final results.

From these tables it will be seen that a change in the character of the perfusing fluid often brings on a marked change in both the minute-volume venous outflow and the amount of reducing substances in this outflow for equal time units. The amount of this change for both factors is expressed in terms of percentage in table 3. In this table the individual observations of all the experiments are segregated according to the characteristics of the perfusing fluid used. In this way one can see better the tendency of the effect of each solution. If the

effect is an increase of the volume outflow or of the sugar-output per time unit, a plus sign is placed before the figure that shows the percentage of this increase; if the effect is a decrease, a negative sign is placed before the figure expressing the change.

With the results so presented one easily sees first that usually wherever the volume outflow is increased there also the sugar output per unit of time increases, and *vice versa* wherever a decrease in one of these factors occurs there also a decrease occurs in the other factor. Whenever this is not the case the figures are bracketed. It will be noted

TABLE 1

Effect of C_H and minimal adrenalin content of a perfusing fluid upon the minute-volume and sugar content of the venous outflow of the terrapin's liver

DATE OF EXPERIMENT—REMARKS	ORDER OF PERFUSION	CHARACTER OF PERFUSING FLUID		MINUTE-VOLUME OUTFLOW, IN CC.	MGM. OF REDUCING SUBSTANCES IN 10 MINUTES OUTFLOW†
		pH index	Adrenalin (1: 10^9)		
*January 26, 1922.** Pres., 13 cm. of water; temp., 20°C.	1	7.2	+	13.33	7.33
	2	7.2	0	10.1	6.55
	3	7.6	0	3.68	2.61
	4	7.6	+	3.9	0.91
February 16, 1922. Pres., 10 cm. of water; temp., 22°C.	1	7.2	+	8.71	5.51
	2	7.2	0	7.03	4.77
	3	7.6	+	5.00	4.37
	4	7.6	0	9.70	3.88
	5	7.2	0	8.73	6.84

* This liver was removed entirely from animal, and all outflow (including possible lymph) collected.

† Calculated from the analyses of 50 cc. samples.

that out of a total of forty-two observations there are only five decided exceptions two of which were made under very unusual circumstances (see case 3). As the footnote to the table indicates, these exceptions clearly should not be given equal weights with the other observations.

Second, while the change in the sugar output tends to follow in the same direction as that of the volume flow per unit of time, the amount or extent of the change in the two factors do not seem to bear any relation to each other. The period of perfusion was probably too short in most cases to allow the observations on this point to be at all decisive.

Since the effects of these changes of perfusing fluids on the mere volume outflow from the hepatic veins has been dealt with elsewhere

(1) they may be dismissed without further comment except to point out that the results here for the most part corroborate the former findings. We shall therefore focus our attention for the present upon the output of reducing substances.

TABLE 2

Effect of C_H and minimal adrenalin content of a perfusing fluid upon the minute-volume and sugar content of the venous outflow of the terrapin's liver

DATE OF EXPERIMENT—REMARKS	ORDER OF PERFUSION	CHARACTER OF SOLUTION		SECONDS TO COLLECT 100 CC. OF VENOUS OUTFLOW (OBSERVED)	MINUTE-VOLUME OUTFLOW (CALCULATED)	MGM. OF REDUCING SUBSTANCES IN VENOUS OUTFLOW IN 10 MINUTES†
		pH index	Adrenalin (1: 10^9)			
February 18, 1922. Pres., 9.5 cm. of water; temp., 22°C. Each sol. is passed *ca.* 10 minutes before taking samples	1	7.6	+	793	7.6	6.14
	2	7.6	0	646	9.3	10.0
	3	7.2	0	445	13.5	10.13
	4	7.2	+	395	15.2	15.3
	5	7.2	0	463	13.0	12.34
February 23, 1922. Pres., 10 cm. of water; temp., 21°C. Each sol. is passed through liver for *ca.* minutes; 0.05 per cent dextrose is added to each solution	1	7.0	0	266‡	11.27	10.4
	2	7.0	+	269	11.1	14.3
	3	7.0	0	300	10.0	12.6
	4	7.6	0	769	3.9	7.7
	5	7.6	+	1682	1.8	2.0
	6*	7.0	0	408	7.3	21.3
March 4, 1922. Pres., 9.5 cm. of water; temp., 22°C. *Ca* 0.05 per cent dextrose is added to each sol. Each sol. is passed to collect 70 cc. before collection of samples begins	1	7.6	0	786	3.81	4.6
	2	7.6	+	900	3.33	3.5
	3	7.6	0	780	3.84	6.3
	4	7.0	0	218	13.75	16.1
	5	7.0	+	383	7.84	10.1
	6	7.0	0	454	6.60	3.8
	7*	7.0	0	430	6.97	9.0

* This perfusion had no sugar at the inflow.

† Calculated from the determinations of the contents of 50 cc. samples.

‡ The figures from here on in this column are the times to collect 50 cc. only instead of 100 cc.

The effects of the various perfusing fluids on the sugar-output, as already pointed out, are in general in the same directions as they are upon the volume flow. This fact appears in a more striking way if we

TABLE 3

Showing percentage of change in time-volume venous, outflow and time-weight output of reducing substances by the terrapin's liver. The plus signs before the figures signify increases; the minus signs, decreases

NUMBER OF CASE	CHANGING FROM		PERCENTAGE IN TIME-VOLUME OUTFLOW	CHANGE IN TIME-WEIGHT OUTPUT OF SUGAR
	Perfusing fluid of	To perfusing fluid of		
1	Low pH	High pH	−63	−60
			−61	−39
2	High pH	Low pH	[−10]	+76
			+45	+ 1.3
			+258	+155
3	Low pH	Low pH + adrenalin	+12.6	+50
			± 1.0	+38
			[−43]*	[−38]*
4	High pH	High pH + adrenalin	[+6]	−65
			−54	−74
			−12.5	−24
5	Low pH + adrenalin	Low pH, no adrenalin	−29	−11
			−29	−12
			−14.4	−19.3
			−10	−12
			−16	−64
6	High pH + adrenalin	High pH, no adrenalin	+62	[−11]
			+22	+63
			+15	+80
7	Low pH, no adrenalin	High pH + adrenalin	−15	−8
8	High pH + adrenalin	Low pH, no adrenalin	+300†	+958†

* These results were obtained upon changing from the solution that gave the enormous dilatation noted above in case 2. See numbers 4 and 5 of experiment of March 4.

† For this remarkable result see number 6 of the experiment for February 23, especially footnote.

tabulate the actual sugar analyses from the various perfusates according as to whether adrenalin is present or absent and as to whether the pH is low or high.

This has been done in table 4 for all the experiments. The figures here given are mean values wherever the same perfusate was tried more

TABLE 4

The mean output of sugar by the liver when perfused with solutions of low and high pH, and with and without minimal amounts of adrenalin present; in milligrams per 10-minute periods

ADRENALIN, 1: 10^9	ABSENT		PRESENT	
pH index	Low	High	Low	High
January 26	6.55	2.61	7.33	0.91
February 2	2.75	2.46	4.32	2.46
February 4	[0.72]*	[3.6]	[1.10]	[1.63]*
February 11	[3.90]*	[5.67]	[2.12]	[3.98]
February 16	5.04	3.8	6.17	4.37
February 18	9.4	7.7	14.1	6.14
February 23†	11.6	7.7	14.3	2.0
February 25†	[13.3]*	[4.7]	[17.3]	[4.28]*
March 4†	9.6	6.3	10.7	3.5
Final mean*	7.17	5.08	9.31	3.52
Final average*	7.49	5.09	9.48	3.23

* The bracketed observations were not included in determining the final values. Those for February 4 and 11 are of doubtful value because the sugar analyses of the samples were delayed in one case for 17 days in the other for 10 days. Whereas in all the other experiments the analyses were made the same day or the day following the experiment. The figures for February 25 are excluded for the reason given in the following note.

† Dextrose to about 0.05 per cent was added to the reservoirs of perfusing fluids in each of these cases. The exact content of dextrose was then determined by analysis and subtracted from the amount found in the venous outflow. In the case of February 25 this correction is not included since curiously they then turn out to be negative; active glycogenesis apparently had taken place.

than once in the same experiment. The figures in the column for any given fluid vary considerably but nevertheless there is a trend of magnitudes that appears at once when we take the mean of the extremes for each column. As the footnote to this table indicates, there is good reason again not to give all the results equal weight. In trying to get a final figure (called "final mean" in the table) for each fluid the results of six out of the nine experiments were considered satisfactory enough

as a basis. The averages of the columns (excluding again the bracketed observations) it will be noted are nearly the same as the means. The trend of these results can be put graphically as is done in table 5 from which the results of the experiments of February 4 and 11 have been excluded. Here the perfusion that is accompanied by the greatest output of reducing substances during the experiment has a symbol of two cross marks (++) in its appropriate column; the perfusion that is accompanied by the next largest amount of reducing substances has a single cross mark (+) in its appropriate column. On the other hand the perfusion that is accompanied by the least output of reducing substances has two negative signs (−−), and that having next to the least output has one negative sign (−), placed each in it appropriate column.

TABLE 5

Minute-weight output of reducing substance by the liver

DATE OF EXPERIMENT	ADRENALIN ABSENT		ADRENALIN PRESENT	
	Low pH	High pH	Low pH	High pH
January 26	+	−	++	−−
February 2	+	−	++	−
February 16	+	−	++	−−
February 18	+	−	++	−−
February 23	+	−	++	−−
February 25	+	−	++	−−
March 4	+	−	++	−−

Since the output of sugar by the liver, from these results, clearly is diminished with the diminished outflow, that is, during the great constriction of the liver vessels, it appears that the changes in the output are primarily due to the minute-volume outflow rather than changes in sugar concentration. If this is the case one would expect the concentrations of the samples collected from the various perfusions to exhibit some approach to constancy. To decide this point the sugar analyses of our experiments have been brought together in terms of mean percentages in table 6. In this table the extremes for each solution for all the experiments are underscored; their means appear at the foot of the columns, the averages of all are given in the last line. In reading the percentages down any one column one must bear in mind that while they are for the same solution still they are for different livers in each case.

To one familiar with the observed blood sugars of various animals (see Macleod, 3a) the percentages of reducing substances, as we have found them may appear to be exceedingly low, and to those not familiar with the modern micro-methods of blood sugar analysis a doubt may arise as to whether such small amounts can be measured at all with any satisfactory degree of accuracy. In regard to the first point we need only remind the reader that the terrapins were hibernating, at least were in a state of inanition and that the livers were thoroughly washed with Ringer's before the samples were collected. The Ringer's itself either had no dextrose added at all or in those cases in which dextrose was added (expers. for Feb. 23 and 25 and Mar. 4) it was only in concentrations of less than 0.05 per cent. In these latter cases

TABLE 6

Percentage reducing substances in the venous outflow from terrapin livers perfused with Ringer's

Adrenalin	Absent		Present	
pH index	Low	High	Low	High
Experiment of January 26	0.0065	0.0071	0.0055	*0.0023*
Experiment of February 2	*0.0028*	*0.0035*	*0.0028*	0.0051
Experiment of February 16	0.0048	0.0088	0.0063	0.0080
Experiment of February 18	0.0075	0.0080	0.0102	0.0081
Experiment of February 23	*0.0108*	*0.0176*	0.0128	*0.0110*
Experiment of March 4	0.0082	0.0142	*0.0138*	0.0104
Final mean	0.0068	0.0105	0.0083	0.0066
Final average	0.0067	0.0098	0.0085	0.0075

samples of the perfusing fluids were analyzed for reducing substances by the same method as was used for samples from the outflow. This number was taken to represent the reducing substance at the inflow, and was subtracted from the percentage found in the outflow. The difference is the figure put into the tables here given. As to accuracy of the method for sugar determination the reader must consult the authors of the method used, Shaffer and Hartmann (2), and also Bang, Maclean, and others whom they cite. Our blanks and controls left no doubt in our minds as to the accuracy and reliability of the analyses.

A word in addition may be said in regard to the results of the experiment for February 25 (see table 4). Here we found the reducing substances in the outflow in every case to be less than the amount in the Ringer's at the inflow. Apparently the glycogenetic processes in

this liver were more active than the glycogenolytic processes. Analysis of the glycogen content of the liver before and after perfusion (which was not done in these experiments) may have shed some light on this case.

There is one other matter that requires a passing comment. It has been stated that the hepatic outflow which we collected was that from the hepatic veins, but that in one experiment the lymph, if any flowed out, was also included in the collected outflow, experiment of January 26. It is noteworthy that the results of this experiment as to minute-volume output are not quite in accord with the rule, but the minute-sugar output agrees with the results of the other experiments. However in the table of *percentages* it will be noted the results disagree with the average not more than do those of any of the other experiments. Lamson (4) used very heavy doses of adrenalin in his experiments, it will be remembered, which seemed to shunt the circulating fluid into the lymphatics. Our minimal doses apparently had less effect on lymph flow.

Before commenting further upon the results in table 6 it will be helpful again to reduce them to the graphic form. This has been done in table 7. The symbols here have the same significance, referring in this case to greater and lesser amounts of reducing substances per 100 cc. of hepatic outflow instead of amounts in minute-volumes as is the case in table 5. From tables 6 and 7 it is at once clear that the concentration of reducing substances in the hepatic outflow is anything but constant whether dextrose has been added to the inflow or not. On the other hand this variation in concentration does not appear to follow very sharply any of the perfusing fluids. However if one inspects the line of mean values of table 6, and the four columns of symbols (table 7), in detail the following points may be noted:

1. In the hepatic outflow for a given terrapin the greatest and least concentrations of reducing substances *tend* to occur when adrenalin is absent, the greatest occurring when the perfusing fluid is more alkaline, the least when it is more nearly acid (table 7).

2. If minimal adrenalin is present in perfusing fluids of low and high pH, *a*, there tends to be less difference on the one hand in the concentration of sugar, at the outflow, between the two adrenalin-containing solutions; and *b*, the concentrations of these latter tend to occupy a middle place between the extremes ot the adrenalin-free solutions.

It would appear then that epinephrin in minimal dosage tends to maintain a more constant concentration of reducing substances in the

hepatic outflow when saline solutions are used for perfusion, opposing the increase that those of higher pH tend to produce on the one hand, and on the other hand opposing the decrease that those solutions of low pH tend to produce.

Recalling now that the hepatic volume outflow is greatest for an adrenalin-containing solution of low pH, and that the minute-output of reducing substances by the liver varies precisely in the same way, being greatest for the low pH + adrenalin solution and least for the high pH + adrenalin solution, we are forced to the conclusion that *the rôle of epinephrin in great dilution is not concerned directly so much with the mobilization of sugar from the liver as it is with determining the amount of fluid flowing through its portal-venous bed.*

TABLE 7

Percentage reducing substance in outflow from liver

Adrenalin	absent		present	
pH index	Low	High	Low	High
Experiment of January 26	+	++	−	−−
Experiment of February 2	−−	−	−−	+
Experiment of February 16	−−	++	−	+
Experiment of February 18	−−	−	++	+
Experiment of February 23	−−	++	+	−
Experiment of March 4	−−	++	+	−

Discussion. Before entering upon even a brief discussion of the general problem of the rôle of the liver in controlling the amount of sugar in the circulating blood, it is important to emphasize the highly restricted conditions under which the present results were obtained. This restriction was employed designedly for it was felt that only in so doing could many of the uncontrolled factors in the problem be eliminated. Accordingly we have so greatly changed the conditions from what may be called normal that in comparing the results with those obtained on practically intact animals one must use great caution at every step.

With the method and material used in this work we have been able to measure directly minute-output as well as percentage content of reducing substances of the hepatic outflow. In experiments upon mammals with the circulation intact this has rarely been done, workers having been content with taking samples from the general circulation or from the portal vein and the vena cava near the entrance of the

hepatic vein from time to time, and determining and comparing the percentage of reducing substances therein. Now it is quite clear that the percentage of a substance may be high and the volume output per unit of time low, so that no increase in the percentage of the general circulation obtains. If in 10 minutes the liver outflow under one set of conditions happens to be 100 cc. and the content of the substance 0.05 per cent, and under another set of conditions the outflow is 70 cc. in 10 minutes with the concentration of the same substance at 0.06 per cent, to argue that under the latter conditions the output is greater is quite false, for indeed a little calculation will show that, although the concentration has increased 20 per cent the actual output is less by 16 per cent. Unless one can actually measure the volume outflow from the liver the method of determining concentrations of substances from mere untimed samples flowing therefrom is obviously wholly unreliable. This criticism is all the more pertinent when one has introduced into the blood, or fluid, flowing through the organ, changes in the C_H or drugs and chemicals that affect the caliber of the blood vessels and thus the capacity of the vascular bed, that is, substances such as histamine or epinephrin. This precaution follows not only from the results of the present investigation but is an unavoidable inference from the works of Lamson, and Lamson and Roca on the blood-volume regulating function of the liver (4).

With this word of caution let us now pass to a short review of some results of the more recent work in this field together with some statement as to the relations of our results to them and to the general problem. For more detailed reviews and discussions, and completer citation of authors the reader may consult Macleod (3a) as well as the other writers herein mentioned.

In the first place it will be of interest to point out that Macleod and Pearce (5), in a study on the volume outflow of blood from the dog's liver under various conditions, showed that when epinephrin was injected into the portal vein the outflow from the hepatic vein was diminished all the way from 0.8 to 57 per cent. If the hepatic artery was ligated the outflow was diminished in some cases, but in two out of seven (28 per cent of all the cases) an actual increase in the outflow was observed. In one of the cases the increase was as much as 17 per cent. In these experiments the concentration of the epinephrin was not less than $2:10^6$. While this dose cannot be considered minimal yet in the two cases of increased outflow observed with the hepatic artery tied off, resulting as it would in increased C_H of the blood

in the liver, one is inclined to suspect a reverse epinephrin reaction as the responsible agent. As to the increase of C_H under such conditions Macleod and Wedd (6) later were able to show that the blood, collected from the hepatic vein when the hepatic artery was tied off, did contain a marked increase in lactic acid and therefore presumably C_H. Curiously enough however in a work of the same year Macleod (7) when making quantitative studies on liver glycogen does not appear to have made simultaneously minute-volume determinations of the hepatic outflow, although the experiments involved marked changes in the C_H of the inflow. Hence one is not certain that there was an actual decrease in the output of sugar. When it is stated that acid or neutral injections "did not reveal any such difference in reducing power of the two bloods," or when one reads "neither did injections of acid or alkali alone cause any difference," one cannot be sure that the minute-output of reducing substance underwent no change. Furthermore, from what has now been said, to try to avoid this error by estimating the volume flow through the liver for one set of conditions from observations made under other conditions would likewise be equally useless.

The general trend of the evidence of many observers however has been toward the view that acid facilitates and increases hepatic glycogenolysis and that alkali not only retards it but actually promotes the reverse process, that is, a storage of sugar in the liver cells and its conversion there into glycogen. According to Elias, and to Murlin and Sweet and others, hyperglycemia follows administration of dextrose together with acid, given either *per os* or intravenously. According to Murlin and Kramer, to Underhill and to Macleod, alkali given by way of duodenum or intravenously to dogs brings on hypoglycemia, and in depancreatized animals decreases both their hyperglycemia and glycosuria. Injection of dextrose plus carbonate into the portal vein of dogs results in lowering of the percentage of reducing substances in the blood taken from the vena cava near the mouth of the hepatic vein (7). In those first attempts to demonstrate the formation of glycogen by the liver from dextrose added to the circulating fluid the failure of Martz and the success of Grube may have been due at least in part to an accidental and uncontrolled C_H of the perfusing media.

Since the pioneer work of Blum it has been known that epinephrin produces glycosuria; Foa and Gatin Gruzewska thought that it also lowers the alkalinity of the blood as well as of the urine. These later observers showed also that the same results followed piqûre; later

workers have indicated that the piqûre effects are really of a nervous nature. Underhill (8) found that if sodium bicarbonate were first administered to dogs adrenalin no longer called forth so much hyperglycemia, and Kramer and Marker (9) found that adrenalin did not increase the glycosuria of deprancreatized dogs if they had just been given sufficient alkali. Bang (10) observed that frogs' livers suspended in Ringer's solution to which adrenalin had been added gave up much more reducing substance than when no adrenalin was present. The C_H of the Ringer's is not given but according to page 19 of the report (10) the solution was made up out of ordinary distilled water and no carbonate or phosphate. It must therefore have been either neutral or even acid rather than alkaline.

In demonstrating the rôle of liver diastase in hydrolyzing glycogen *in vitro*, Langfeldt (11) controlled the C_H of his solutions; his results therefore have the greatest interest. They show *a*, that the action of the diastase in solutions of 0.6 per cent NaCl buffered with phosphates is at its maximum at about pH 6.8, thence falls abruptly toward the lower pH values on the one side, and asymptotically toward the higher pH values on the other side. It is noteworthy that the action is lowest on the alkaline side already at about pH 7.8. If adrenalin in relatively great concentration (1:125000) is added to solutions containing phosphates (but no chloride), sugar formation although at all pH indices markedly augmented, has its maximum value shifted over to pH 7.7. If the concentration of the adrenalin is lowered to $2:10^6$ the maximum sugar formation drops back to pH 7.0; if the adrenalin concentration equals $2:10^7$ the diastatic action is greatest at pH 6.8 again, and the amount of sugar formation is no greater at any pH than in the adrenalin-free phosphate solutions. It is to be regretted that an adrenalin concentration of $1:10^9$ was not used, but still more that the adrenalin was not put in the 0.6 per cent NaCl solutions + phosphate. I quote the percentage hydrolysis from Langfeldt as follows:

pH	PHOSPHATE SOLUTION (FROM TABLE I)	NaCl + PHOSPHATE (FROM TABLE II)	PHOSPHATE + ADRENALIN 2: 10 (FROM TABLE III)
6.8	13.18	27.0	14.2
7.7	5.64	11.4	6.2

While we cannot be certain from the above that the adrenalin in our experiments was too dilute to have any effect upon the diastatic ferment of the liver, yet on the other hand we are justified in be-

lieving that the chlorides and the phosphates we used had an action somewhat similar to this demonstrated in the diastase studies.

If we now look back at our tables 4 and 5 on the minute-output of sugar by the terrapin's liver we may explain the results therein by saying that first, when adrenalin was absent a greater output occurred at low pH for two reasons: *a*, because there was a greater volume of fluid flowing through the liver, see (1) and table 3; and *b*, because phosphate-sodium chloride solutions tend to facilitate or augment the activity of the liver-diastase that was presumably present in the liver cells. On the other hand, second, when minimal amounts of adrenalin are added the output of sugar is further augmented for solutions of low pH, *a*, because the volume flow through the organ is increased by minimal adrenalin; and *b*, because the sodium chloride-phosphate solution presumably has still the same augmenting action as it does without adrenalin. The adrenalin itself may or may not have an additional augmenting action. That the solutions of high pH with adrenalin present show even a smaller output of sugar than do those without adrenalin is explicable from the fact that the adrenalin augments the constriction already brought on by the OH-ions. The outflow of fluid per unit of time being cut down, the output of sugar is likewise cut down. It may be shown later that dilute adrenalin in solutions of high pH has a retarding action on the glycogenolytic action. The diminished irrigation surely will diminish the flushing out of preformed sugar from the liver cells.

Turning now from minute-output to percentage content of sugar in the hepatic outflow (tables 6 and 7) the following comments may be made: In the first place the differences in percentages do not invariably follow change of pH in the same experiment either with or without minimal adrenalin being present, and while there is a tendency for them to do so even then the difference is not always well-marked. The reason for this result is easily understood when one considers the very great changes the perfusing fluids have upon the minute-volume outflow, and that while these changes under the proper conditions invariably go in the same direction they do not always occur with equal intensities (see table 3). A haphazard variation then in the percentage of sugar at the outflow is what one would expect rather than a regular variation. That there is a tendency toward some regularity however in percentage oscillation with the pH, as the tables show, may be explained as follows: *a*, In the case of the adrenalin-free solutions the percentage of sugar tends to be higher for the solutions of high pH be-

cause these so constrict the blood vessels that a smaller volume of fluid comes in contact with the liver cells. There is thus time for the diffusion process to carry out more of the preformed sugar and thus raise the concentration in the blood stream even though the process of glycogenolysis is greatly reduced. The diminished flow of fluid however reduces the rate of gaseous exchange and thus causes some asphyxiation. This process lowers the pH and will counteract the retarding effect upon the ferment action. Diffusion will do the rest. This latter effect while tending to raise the *percentage* of sugar at the outflow for high pH solutions still is not great enough to maintain the *minute-output* at the high-level that obtains with solutions of low pH (table 5). *b*, In the case of the solutions containing minimal adrenalin we have noted a *tendency* for the percentage of sugar in the hepatic outflow to be slightly greater for the solutions of low pH than for those of high pH. This is only a tendency again and that it is not invariable is again explicable for the same reason given above,—the great variation in the amount of vasodilatation and -constriction caused by the same agents. It will be remembered adrenalin-containing solutions of low pH cause even greater minute-output (table 5) than do adrenalin-free solutions, in amounts of fluid as well as sugar. This we have explained above. The lower pH favors the diastatic action and the epinephrin opens up the blood vessels (no doubt the venules) wider allowing more liver cells to be irrigated. Langfeldt's observations that dilute adrenalin has no effect on the diastatic action, as pointed out above, may not apply in these experiments. Indeed we are inclined to think that further experimentation with the diastatic ferment will show that dilute adrenalin increases the diastatic ferment action in low pH solutions over and above the effect of the mere NaCl-phosphate agent. At any rate that the percentage of sugar in the outflow for low pH solutions tends toward being greater than for those of high pH if adrenalin is present warrants such expectation. The tendency is due doubtless to the increased glycogenolysis and to an increased flushing out of the cells of preformed sugar. In any event the final result is another reverse action of epinephrin brought on by a change in the hydrogen-ion concentration.

SUMMARY AND CONCLUSION

Comparing the effects of perfusing terrapin's liver with Ringer's solutions buffered with phosphates, some of which are neutral or slightly acid and others a little more alkaline than normal blood, we find:

1. That the volume outflow per unit of time in general is greater for the solution of lower pH, less for the solution of higher pH. This confirms the observation of Snyder and Martin (1).

2. That the output of reducing substances per unit of time in the venous outflow is greater for the solution of lower pH, less for the solution of higher pH. The mean difference of five observations is as much as 78 per cent.

3. That when minimal effective amounts of adrenalin-Takamine are added to the perfusing fluids of low and high pH indices, *1*, the minute-volume outflow *a*, for solutions of higher pH is markedly less, and *b*, for solutions of lower pH is markedly greater, than for adrenalin-free solutions of equal pH values. (This confirms the further observation of Snyder and Martin (1) of an epinephrin reversal for the portal venous system of the terrapin effected by a change in hydrogen-ion concentration.) *2*, the minute-weight output of reducing substances *a*, for solutions of higher pH is markedly less (mean of 6 observations, 35 per cent) and *b*, for solutions of lower pH markedly greater (mean of 7 observations, 30 per cent) than for adrenalin-free solutions of equal pH values.

4. This latter observation would point to a specific "epinephrin reversal" for the process of hepatic glycogenolysis, but analysis of the conditions prevailing leads the authors to conclude that the great variations in output of reducing substances produced by minimal amounts of adrenalin are to be ascribed more to the direct effects of the drug on vascular caliber and hence upon intensities of irrigation rather than directly upon the glycogenolytic processes of the liver. The latter processes are however indirectly affected because of the greater number of cells becoming exposed to the chloride-phosphate contents (Langfeldt) of the perfusing fluids during vasodilatation, and fewer cells becoming so exposed during vasoconstriction.

5. The results of the present investigation show that the determination of mere percentages of reducing substance in untimed samples of liver outflow furnishes no reliable data concerning the absolute amount of that substance delivered up to the general circulation (compare tables 4 to 7). In problems of hepatic glycogenesis or glycogenolysis minute-output determinations appear to be indispensable. Furthermore the flow from the lymph vessels, as well as blood vessels, probably should be included in investigations of this kind.

6. As to the evidence that has accumulated from experiments upon mammals and man concerning the action of acids and alkali upon the

output of sugar by the liver, our results give conclusive support to the view that acid increases and alkali reduces the output. Our method of experimentation was designed to keep a greater number of the variable factors under control. We thus are enabled to add a word concerning the probable *modus operandi* of the sugar regulating action of acid and alkali in the higher animals. The action appears to be two-fold. In the first place, in keeping with observations that began with Gaskell on the effect of acid and alkali on blood vessel walls, as well as our own, it becomes highly probable that in the mammal's liver vasodilatation may be elicited by acid and vasoconstriction by alkaline. This may involve vast changes in mere volume-flow bringing now greater now lesser numbers of cells into action. In the second place the chloride-phosphate contents of the circulating fluid (and blood contains them both) as Langfeldt has shown may augment the activity of the liver diastase, more when the fluid is slightly acid or neutral, less when it is alkaline. That the sugar output under these conditions also becomes greater to acid and less to alkaline circulating fluids thus becomes an inevitable consequence.

7. Concerning the glycogenolytic action that has been ascribed to epinephrin upon the basis of mammalian experimentation and clinical observations, we can only say that in the terrapin the minimal vascular effective dose (the only dose we used) so far has revealed no direct action upon the liver cells. There is however a well-marked indirect action, which we have shown to be due to the vascular effect rather than any other. Our experience, however, indicates that the whole question of the rôle of epinephrin in hepatic function needs further investigation, a work that we hope to carry out in part in the near future.

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THE EFFECT OF INTRAVENOUS SODIUM BICARBONATE ON INTESTINAL MOVEMENTS

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The fact that sodium bicarbonate administered intravenously has a marked effect upon the movements of the gastro-intestinal tract of dogs was first forcibly brought to our attention while doing some experimental work reported in a previous communication (1). A study was being made of the acid-base balance relative to the movements of the intestinal mucosa. Sodium bicarbonate was found to be a vigorous stimulant of the intestinal mucosal musculature. At the same time movements of the outer musculature were set up, so stormy at times that it was almost impossible to observe the mucosa with the binocular microscope.

In searching the literature at our disposal we have found very little reference and discussion relative to this reaction. The various texts on pharmacology usually mention that large doses of sodium bicarbonate by mouth may lead to purging, presumably by irritant action. Within recent years clinicians to some extent have employed the intravenous method of administering bicarbonate in acidosis. We have gone over a number of reports on its usage in this manner but have found no mention of any signs of increased intestinal activity. Personal discussions with clinicians have brought out no references to any such reactions.

Howell (2) made a study of the effect of sodium carbonate on blood pressure in shock. He found it to be a good cardiac stimulant. Dawson (3) extended Howell's work, but used sodium bicarbonate. Howell attributed the pressor effect to the alkalinity. Seelig, Tierney and Rodenbaugh (4) found that sodium bicarbonate increased the amplitude of the heart in shock animals. They considered the effect as due to a direct action upon the heart. These references have no direct bearing

on the response of the musculature of the gastro-intestinal tract to carbohydrates, but they show that both carbonates and bicarbonates affect the activity of a part, at least, of the smooth musculature of the body.

Collip (5) found that sodium bicarbonate introduced into the subarachnoid space produced marked evidence of medullary stimulation. Intravenous injections were without effect. All of his animals received morphine before anesthesia.

Hatai and Hammett (6) found that sodium bicarbonate invariably produced an increase in tonus in segments of the small intestine of the albino rat. They found that a preliminary stimulation of the splanchnics made the strips less excitable, but that a preliminary administration of epinephrin did not affect the reaction.

Alvarez (7) could demonstrate no increase in the rate of the rhythmical intestinal movements after sodium bicarbonate, but did demonstrate an increase in the amplitude.

While our investigations were in progress a paper by Hammett and Nowrey (8) and one by Hammet (9) appeared in which the authors claimed that the effect of sodium carbonate and bicarbonate on the segments of the small intestine of the albino rat were not due to the sodium ions, carbonate ions, to the sodium-calcium ratio, but to an increase in the OH ions. They also state the effect to be brought about through the intrinsic nervous mechanism of the intestine.

The fact that the addition of sodium bicarbonate to physiological salt solution is advantageous has been known for a long time. Ringer's, Locke's, and Tyrode's solutions all contain enough bicarbonate to maintain the reaction slightly on the alkaline side of neutrality.

The present study was undertaken for the purpose of ascertaining more fully the type of the motor response of the small intestine of the dog to sodium bicarbonate, the factors affecting it and the mechanism involved. The work was done on the small intestine in intact animals under ether and after decerebration, and with isolated segments suspended in oxygenated saline. On the whole, the sharpest reactions were obtained from the decerebrated animals, as compared with those etherized. The isolated segments also reacted well. The movements and changes in tonus of the intestine in the intact animals were recorded by the balloon-tambour method. The balloon was distended with water at a pressure of about 35 cm. This was found to be the most satisfactory pressure. The strip movements were recorded in the usual way, suspended in oxygenated saline, fixed at one end and attached to a muscle lever by means of a silk cord at the other end. A study was made of the changes in both the circular and the longitudinal muscles.

The sodium bicarbonate was used in strengths of 2.5 per cent, 4 per cent, and 6 per cent, 50 cc. being the dose usually employed. Merck's "C.P." sodium bicarbonate was used in the majority of experiments. Most of the other common brands on the market were tried. The reason for this diversity grew out of the fact that the earlier results were obtained from a sample of bicarbonate obtained from a large laboratory stock bottle, the label not indicating the purity or source. It was thought that possibly some impurity might account for the reaction. We were, however, not able to demonstrate any significant differences in reactions.

The bicarbonate solutions were prepared for use in a variety of ways. Practically all preparations when first put into solution were slightly alkaline to phenolphthalein. In order to imitate as nearly as possible the procedure in the administration of bicarbonate to the human, outlined by Howland and Mariott (10), bottles of 500 cc. capacity were filled with the solution, saturated with carbon dioxide, tightly stoppered, then sterilized in an atmosphere of CO_2 in the autoclave at 15 pounds pressure for 15 minutes. With a few exceptions these preparations were acid to phenolphthalein. Results with these solutions were compared with those from solutions not altered in any way, with solutions to which definite amounts of hydrochloric acid had been added, and also with solutions to which sodium hydroxide has been added to increase the alkalinity. There were no distinct or constant differences which could be ascribed to any changes brought about in the preparation of the material, except that the intensity of the reactions ran somewhat parallel with the amount of available bicarbonate. After this point was determined the bicarbonate was merely dissolved in water and warmed to the body temperature of the animal.

Characteristics of the reaction of the small intestine to sodium bicarbonate. The most constant effect observed and recorded was an increase in tonus. This was true both in the intact animals and in the strip experiments. Next in prominence was an increase in amplitude, and finally an increase in rate. The increase in rate was not constant, neither were we able so to control conditions so as to be able to produce a variation in rate. Occasionally an animal was obtained which was very refractory.

The balloon was as a routine placed into the lower duodenum or upper jejunum. All the levels from the pyloric valve to the ileocecal opening were used. The reactions were qualitatively the same, but on the whole the upper end of the small intestine was the most

reactive. The large intestine was also tested and invariably gave an increase in tonus but very little rhythmical movement (figs. 1 and 2).

The type of movement most commonly recorded and observed was the local segmental movement. Occasionally true persitalsis was set up (figs. 3 and 4).

The reaction lasted for some minutes. In many instances the tonus of the intestine remained higher than before the injection for more than an hour. In some instances rhythmical movement persisted during this whole time. The excitability of the intestine to autonomic drugs usually used in the study of motor activities of the intestine was increased by bicarbonate.

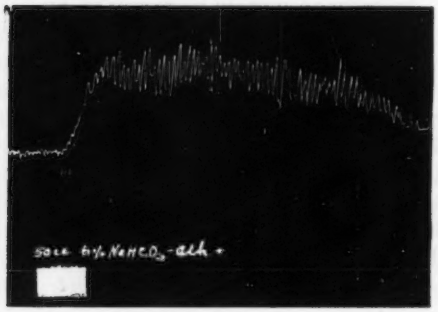

Fig. 1

Fig. 2

Fig. 1. Record from the duodenum following the intravenous injection of 50 cc. 6 per cent $NaHCO_3$, showing considerable increase in tonus and small segmented movements.

Fig. 2. Record from the colon following the injection of 50 cc. 6 per cent $NaHCO_3$, showing marked increase in tonus.

Effect of repeated injections of sodium bicarbonate. In intact animals under ether anesthesia or decerebrated, the reactivity to repeated injections of bicarbonate gradually diminished. This also held true for suspended strips, but in this case, if the surrounding fluid was drawn off and the strip washed, good reactivity was again established. The first reaction was nearly always the most complete. The tonus reaction was the last to disappear (figs. 5 and 6).

Through what mechanism does the bicarbonate act? Is central stimulation of the vagus mechanism involved? The evidence points rather conclusively to a negative answer. In a number of experiments the

vagi were both cut during the height of the reaction. The record was not altered. In another series the vagi were cut before the administration of the drug, the usual type of reactions following the injection of the bicarbonate. In another series the medulla was pithed. This did not affect the reaction. In a number of animals there was some manifestation of medullary stimulation, indicated by increased respiratory effort, vomiting, and a sustained rise in systemic blood pressure. The vomiting was the most pronounced evidence, the respiratory and

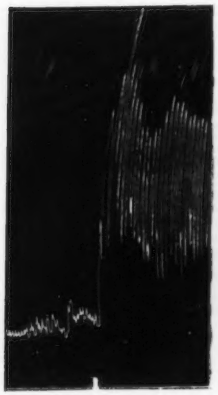

Fig. 3

Fig. 4

Fig. 3. Record from the upper jejunum following the intravenous injection of 50 cc. of 6 per cent $NaHCO_3$, showing increase in tonus and pronounced segmental movements.

Fig. 4. Record from the upper jejunum following the injection of 50 cc. 6 per cent $NaHCO_3$, showing peristalsis.

circulatory changes being slight and not accompanied by a diminished heart rate, but a slight increase, except during the vomiting paroxysm when there were short periods of cardiac slowing. These results indicate that whatever, if any, impulses pass from the medulla to the intestine by way of the vagi, they are not the determining factor in the bicarbonate reaction.

Another line of evidence against central vagus action is the fact that isolated segments reacted just as well and in a similar manner as the intact intestine in the animal.

Is the reaction due to peripheral vagus stimulation? The evidence collected bearing upon this point was obtained through the use of atropine in combination with the sodium bicarbonate. One series of experiments was carried out in which atropine was injected into the animal during the height of the reaction. The results from these tests were not uniform. In about half of the cases the atropine considerably diminished the tonus of the intestine, often to the level before the injection of the sodium bicarbonate. In the other instances the tonus was not affected (figs, 7 and 8). In a few instances the rate of

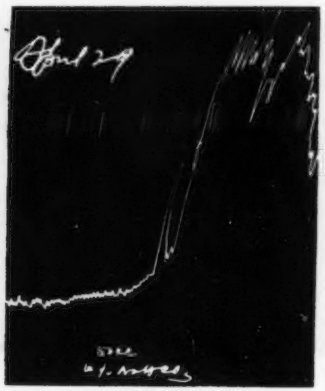

Fig. 5

Fig. 6

Fig. 5. Record from the upper jejunum following the intravenous injection of 50 cc. 6 per cent $NaHCO_3$. First injection.

Fig. 6. Record from the same animal and level as shown in figure 5. Fifth injection of 50 cc. 6 per cent $NaHCO_3$.

contraction was slightly diminished. The amplitude was more variable but not to any marked degree. The same results were obtained with the intestinal segments suspended in oxygenated salt solution.

In another series of experiments the atropine was administered before the bicarbonate. The vagi were stimulated electrically to ascertain the depressing effect upon the endings, and in a few instances pilocarpine was injected. The bicarbonate was not injected until the ordinary methods of vagal stimulation failed to produce any reaction upon the heart and intestine. After this point was reached bicar-

bonate brought about the usual reaction, in fact, some of the sharpest increases in tonus and in movement obtained during the course of all the experimental work were obtained in this series.

These results would indicate that possibly stimulation of the vagal endings may enter in as a factor, but it is not the main factor.

Is the reaction due to the stimulation of the plexus of Auerbach? The work on this point was based upon the assumption that nicotine acts upon the intrinsic nervous mechanism of the intestine in the same

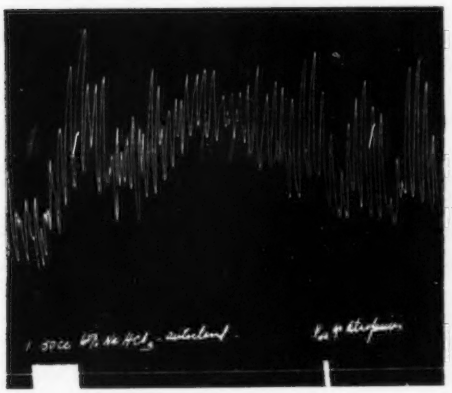

Fig. 7

Fig. 8

Fig. 7. Record showing the effect of following an injection of $NaHCO_3$, with 1/40 gr. atropine sulphate. No marked effect.

Fig. 8. Record from upper jejunum. The marked rhythmical movements followed an intravenous dose of 50 cc. 6 per cent $NaHCO_3$. The atropine brought about considerable fall in tonus, but little or no change in the rate and character of the movements.

manner as upon the extrinsic, namely, first stimulation, then paralysis of the ganglia. In this case as in the former the experiments were divided into two series, one in which the nicotine was administered during the height of the reaction, and the other in which the nicotine was administered before the bicarbonate. The nicotine was given until stimulation of the vagi produced no cardiac inhibition.

When nicotine was given during the reaction there was an abrupt diminution in tonus and movement, followed very shortly by a rapid recovery, often ending in greater tonus and more vigorous movement

than before. The administration of the nicotine before the bicarbonate did not in any way modify the reaction to the bicarbonate.

The same results were obtained from the strips, although in this case we were not able to test out the extent of nicotine paralysis. On the whole, however, the concentration of nicotine reaching the intestine was greater in these instances than when injected into the circulation of the living animals.

These results would indicate that the reaction is not due to a stimulation of the enteric plexus cells.

Is the reaction due to a stimulation of the muscle directly? The evidence on this point is somewhat indirect. We stated earlier in this paper that the reactivity of the gut gradually diminished after repeated administration of the bicarbonate, finally losing all reactivity. When this point was reached pilocarpine was no longer effective, but barium was. This would indicate that whatever if any influence manifested by the vagus mechanism is involved, had been eliminated, and also that the muscles themselves were still irritable.

Bearing upon this point is the character of the reaction to epinephrin when injected during the height of the reaction. Epinephrin brought about a sharp diminution in both tonus and movement which was much more lasting than that after nicotine (fig. 9). It is usually assumed that epinephrin produces very little if any change in the activity of smooth muscle when that activity is due to some stimulant acting directly upon the muscle.

The evidence as a whole indicates that the reaction of the small intestine of the dog to intravenous sodium bicarbonate is due to some factor acting upon the peripherial portion of the intrinsic nervous mechanism, possibly on the myoneural junction.

Miscellaneous points and observations bearing on the reaction. It has been our constant experience that large animals gave more constant and uniform reactions than small ones. We also obtained better reactions in fed than in starved animals.

Some comparative work was done. The small intestines of rabbits, cats and guinea pigs were used. The reactions were similar in character in all the animals tested, but different somewhat in detail. Cats proved most satisfactory for strip work, mainly in the matter of length of time the strips could be kept alive. Rabbits' intestine gave the sharpest reaction when fresh, but we had difficulty in keeping them reactive during a working period, usually 2 to 3 hours. Guinea pig's intestine was the least reactive showing, however, considerable tonus response, but little in rhythmical movement.

The care of the strips before suspension seemed to be a matter of importance. The animals were killed by a blow upon the head, and the small intestine removed as quickly as possibly. The intestines were then dropped into physiological saline at room temperature. Under these conditions they manifested considerable activity, gradually diminishing until they became quiet and relaxed. Segments were then cut and suspended in warm, 38°C., oxygenated salt solution. They soon began to manifest a rhythmical activity of small amplitude. The administration of the bicarbonate at this stage sets up a sharp reaction.

In another series the intestines were removed from the animal as quickly as possible after its death and placed into iced salt solution. The intestines in these instances manifested but little activity but passed into a state of hypertonus and remained so. Strips taken from these intestines and suspended in warm oxygenated saline almost immediately relaxed somewhat and began to manifest vigorous activity. Bicarbonate added during this period of activity brought about very little, often no change in the activity. When a change did occur it was in the direction of augmentation. If these strips were allowed to beat without the addition of bicarbonate they gradually wore out and all activity ceased. Bicarbonate at this point did not revive them, or at best produced only a short period of slightly increased tonicity.

The response of the longitudinal musculature is more marked and more constant than that of the circular muscle.

Several experiments were tried on the muscularis mucosae. The outer musculature was stripped off and the remaining cylinder was mounted with diagonal attachments, the same technique as used by Gunn and Underhill (11). The addition of bicarbonate brought about a marked increase in tonus but we were not able to record any rhythmical movements. Epinephrin in this case gave a reaction identical with the bicarbonate.

After the injection of bicarbonate and synchronous with the sharp reaction on part of the intestine, there is an abrupt fall of blood pressure not due to diminished heart rate. Following this fall the blood pressure rapidly rises to a level slightly above the normal. At the same time the heart rate is increased (fig. 10).

The effectiveness of bicarbonate is diminished by the local application of cocaine.

The intensity of the reaction runs somewhat parallel with the amount of the bicarbonate injected.

The injection of acid into the circulation and its introduction into the oxygenated fluid diminishes the reaction or stops it entirely, the intensity of the effect apparently being dependent upon the relative amounts of acid and alkali used. Work is in progress on the question of the acid-base relations relative to this reaction.

Discussion. Very little need be said relative to the type and the constancy of the reaction of the intestine of the dog to sodium bicarbonate. Out of a total of about fifty animals which we were able to

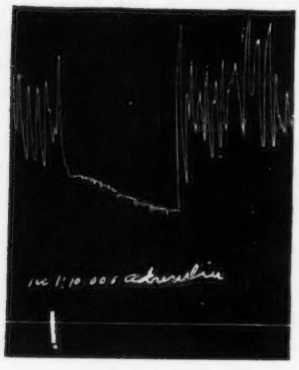

Fig. 9

Fig. 10

Fig. 9. Record from the upper jejunum following the injection of 50 cc. 6 per cent $NaHCO_3$, followed by 1 cc. of 1:10000 adrenalin. The bicarbonate was injected at a point corresponding to about one inch to the left of this record.

Fig. 10. Upper tracing, record from the upper jejunum. Lower tracing, carotid blood pressure. Record following the intravenous injection of 50 cc. 6 per cent $NaHCO_3$.

prepare and maintain under conditions suitable for experimentation only four or five were refractory to the extent that no effect whatever was noted after giving the drug. This shows a constancy as great or greater than can be obtained from the use of the various autonomic drugs such as pilocarpine, atropine and epinephrin.

An increase in tonus is the first phase of the reaction to appear, the most constant, and the last phase to disappear. We have never been able to control the onset of peristalisis but have observed that when it

does set in, it is late in the reaction and after the intestine has relaxed somewhat. We have also constantly observed that if the bicarbonate is administered while the tone of the intestine is great no significant change is brought about in the character of the movements. The suggestion is that the tonus effect is primary and that the local rhythmical and the wave movements are secondary.

The two main points arising out of the observations recorded above are, what part of the sodium bicarbonate is responsible for the reaction, or what change is brought about in the body fluids and tissues which could serve as a stimulus, and what is the point of attack of the stimulus?

The data presented are not sufficient to answer the first question. Our results would warrant the statement that there is a parallelism between the intensity of the reaction and the amount of the drug given the animal. Hammett states that his evidence points to an increase in OH ions as the stimulating factor. We have also observed that the introduction of acid into the circulation of the animal, or into the fluid surrounding a suspended strip, during the reaction to bicarbonate diminishes or abolishes the reaction. With this possibility in mind we prepared bicarbonate solutions of varying strengths and alkalinities, as indicated in the preceding pages of this paper. The results were not definite and clear enough to warrant an unqualified statement that there is a parallelism between the reaction of the intestine and the alkalinity of the fluid used. Seelig and his co-workers claim that they could not account for the effect of sodium bicarbonate on the heart on the basis of alkalinity. To our knowledge none of the workers quoted made a careful study of the changes in hydrogen-ion concentration relative to their reactions, and consequently may have overlooked changes which they could not detect by the methods employed.

The possibility of an increase in CO_2 in the blood and tissues must not be overlooked. The evidence at hand points against this substance as being the stimulus. In a number of animals we introduced CO_2 into the inspired air. Marked respiratory and circulatory changes were observed but no constant or significant reaction on part of the intestine. In a number of animals acid was introduced into the vascular bed after the bicarbonate. Enough gas was liberated in several cases to produce the death of the animal, but brought out no intestinal reaction.

One might also think of a disturbance in the sodium-calcium factor as the cause. Sodium and calcium are antagonistic. An excess of calcium is depressant to most nervous and muscular functions. An excess of sodium leads to hyperactivity. That the sodium-calcium

balance is not responsible is evidenced by the fact that the injection of a soluble calcium salt during the bicarbonate reaction does not modify it unless the dose be very large. Again the injection of the chloride or sulphate of sodium in quantities comparable to the amount of bicarbonate used does not call forth any definite and constant intestinal reaction.

Relative to the point of attack, it is quite clear that the action is peripheral, and that in the main it lies beyond the terminations of the vagus nerve, also beyond the ganglion cells of the enteric system. In making this statement we are not unmindful of the fact that there is considerable difference of opinion as to the terminations and interrelations of the extrinsic and intrinsic nerve elements in the intestinal wall.

It is clear that central vagus stimulation is not a factor of importance. Based upon the results of the use of atropine with the bicarbonate it appears that peripheral vagus stimulation may be a contributing factor, but not the determining one. If we are to hold with Gaskell that the plexus of Auerbach is the terminal vagus mechanism then after atropine there is no reactive point left except the muscle itself, or the less definite myoneural junction. If on the other hand the enteric system is ultravagal, then the bicarbonate may act on any point of it. Since bicarbonate is active after nicotine, and assuming that nicotine paralyzes the ganglion cells of the enteric system, the point of action is again localized peripherally on the endings, myoneural junction, or the muscle.

The argument that the bicarbonate does not act directly upon the muscle is based upon the following observations: the muscle is reactive to barium after it ceases to react to bicarbonate; concentrations of cocaine necessary to abolish the bicarbonate reaction leave the muscle reactive to barium, epinephrin promptly relaxes the muscles of the intestine after bicarbonate, which is usually assumed not to be the case if the reaction is due to direct muscle stimulation; the vascular reaction is not one of pure muscle stimulation.

These arguments are not absolutely conclusive. Barium may be a much more vigorous stimulant than bicarbonate, thus exciting the muscle after it has ceased to respond to the alkali. The main reason for not accepting this is that after the muscle ceases to respond to the ordinary experimental dosage of bicarbonate, much larger doses also have no effect. It does not appear to be merely a matter of relative intensity of stimulation.

In an earlier communication the view was expressed that the enteric system is a complete local nervous system, and also presented evidence

which questioned the notion that nicotine paralyzes the ganglion cells of the plexuses of Meissner and Auerbach. If it does not, then we have no evidence which would eliminate the plexus cells as the point of attack. Whatever the facts relative to nicotine and the enteric cells, it seems clear that the main point of attack is beyond the extrinsic terminations.

There is no evidence of any selective action of bicarbonate on the plexus of Meissner or Auerbach. Bicarbonate stimulates both the outer and the mucosal musculature of the intestine. In a previous communication it was pointed out that stimulation of the splanchnics calls forth a motor reaction on part of the intestinal mucosa, and it is also suggested that the plexus of Meissner is physiologically a terminal splanchnic reflex mechanism. In the same way the plexus of Auerbach is a terminal vagus reflex mechanism. The assumption is that sodium bicarbonate stimulates both mechanisms. The splanchnic mechanism is inhibitory to the outer musculature. It was pointed out in the paper just referred to that mechanical stimulation of the intestinal mucosa under certain conditions brings about a relaxation of the outer intestinal musculature. It would seem then that the end result would depend upon which factor predominates. By the use of epinephrin the inhibitory effect would be intensified and the end result be relaxation. This might explain the observation of Hammett that previous stimulation of the splanchnics in the white rat diminished the susceptibility of the duodenum to carbonate stimulation, and also the cases of our own refractory dogs, inasmuch as we were not able to entirely control the amount of preliminary excitement.

The idea expressed by Bayliss (12) that the addition of bicarbonate to saline serves to neutralize the acids formed in the metabolism of the tissues under observation must not be overlooked. This idea is emphasized by our experience with isolated segments. We were able to get the sharpest reactions from segments which were kept for some minutes in saline at room temperature, thus allowing time for the accumulation of waste products, yet having no efficient mechanism for maintaining a suitable alkalinity.

We have had no opportunity for observing the intestinal motor reaction in the human during and after intravenous bicarbonate. Our experimental dosage has been within the limits of those usually employed by clinicians in acidosis. If the reaction in the human under the conditions of its administration is similar to that in our experimental animals, it is obvious that in some cases it may do considerable harm. One

might wisely hesitate to use it in a case where the intestine has recently been sutured, or where there is danger of rupture from other causes, and in cases of localized peritonitis.

SUMMARY AND CONCLUSIONS

1. The intravenous injection of 50 cc. of 2.5 per cent, 4 per cent, and 6 per cent sodium bicarbonate into dogs calls forth a vigorous motor reaction of the small intestine.

2. The most constant feature of the reaction is an increase in tonus. Frequently local rhythmical movements appear, and occasionally true peristalsis.

3. The intensity of the reaction runs somewhat parallel to the dosage.

4. The reactivity of the intestine is diminished after several successive administrations.

5. The reaction in some cases is diminished by atropine, in others not. Epinephrin temporarily abolishes it.

6. The point of attack appears to be peripheral in the enteric nervous mechanism.

7. The possibility of harm, in the intravenous use of bicarbonate clinically, is pointed out.

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COMPARATIVE STUDIES OF DIGESTION

I. The Digestive Enzymes of Coelenterates[1]

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Our knowledge concerning the digestive enzymes of coelenterates is very limited. The paucity of data in this regard may be appreciated readily on examining the review of Ernst Weinland (1) as well as the earlier reviews of the subject by von Fürth (2) and Hèdon (3). Weinland has prepared a chart in which the digestive enzymes of the invertebrates, so far as they are known, are tabulated. The portion dealing with the coelenterates shows that proteolytic digestion of a few proteins has been observed in these forms by Frédéricq (4), Krukenberg (5), Chapeaux (6), Mesnil (7), Nagel (8), Hamann (9) and Jordan (10). Similarly a rennitic enzyme has been detected in actinia by Mesnil and in Holothuria tubulosa by Cohnheim (11). Digestion of olive oil and monobutyrin in actinia has been reported by Chapeaux and confirmed by Willem (12) and Mesnil. Weak diastatic activity in actinia has been observed by Chapeaux and Mesnil. All of this work was entirely qualitative in nature.

The present investigation is limited to two species of coelenterates which have not been studied hitherto. The first is a large medusa described by Agassiz (13) as Stomolophus meleagris. The second species is the siphonophore Physalia arethusa, or as it is commonly known, the Portuguese-man-of-war. The only experiment which has been recorded concerning digestion in Physalia is that of Bigelow (14) who observed the disintegration of fish meat when placed in contact with the siphons of the Portuguese-man-of-war.

In determining the nature of the digestive enzymes occurring in the two coelenterates, we tested for proteolytic, lipolytic, diastatic

[1] The data in this paper are taken from the thesis presented by Meyer Bodansky for the degree of Master of Arts, University of Texas, 1922.

and rennitic action, as well as for inulinase, invertase, maltase, lactase and raffinase.

Preparation of tissue suspensions. In making enzyme preparations of Stomolophus meleagris (jellyfish) and Physalia arethusa (Portuguese-man-of-war), fresh specimens were used. These were obtained from the sea or immediately after being washed on shore. By transversely cutting the disk of the jellyfish at the level of the gastric cavity, the mesenteric filaments were exposed. These were separated and removed from the gonads by means of fine forceps. The washed filaments were ground with sand to a mash, and the latter diluted with water. Tissue suspensions prepared in this way were fairly homogeneous, and could be pipetted quite readily. The siphons of the Portuguese-man-of-war were separated as much as possible from the tentacles and other tissues, and were thoroughly macerated with sand. As in the case of the jellyfish, the mash itself was used in some experiments, whereas in others, diluted suspensions were employed.

Proteolytic enzymes. For the identification of the proteolytic enzymes in the coelenterates, we followed the procedure employed by Dernby (15) in his experiments on autolysis. For this purpose, gelatin solutions of definite concentration are digested at 37°C. by the tissue suspensions. At stated intervals measured portions of the digests are removed. The progress of digestion is determined by cooling these portions in an ice bath for 15 minutes and noting the degree of liquefaction. As digestion progresses, the gelatin is liquefied and does not solidify completely on cooling, or if the digestion is sufficient, it may fail to solidify altogether. Dernby expresses the approximate degree of digestion by means of the following scale of seven numbers.

0 = Completely solid
1 = Solid, but small pieces may be torn off by strong shaking
2 = Solid, but the surface moves somewhat when tubes are shaken
3 = Soft
4 = Half liquid
5 = Almost liquid
6 = Entirely liquid

As in the work of Dernby, gelatin solutions of varying hydrogen ion concentration were subjected to the action of equal quantities of an enzyme preparation, and the progress of digestion determined at definite intervals. In this way it was possible to estimate, with a fair degree of accuracy, the optimum hydrogen ion concentration at which digestion occurred. Our experience confirms Dernby's statement

that this affords a valuable method for the identification of peptic and tryptic hydrolysis.

In table 1 are recorded the results of a typical series of experiments with the siphons of the Portuguese-man-of-war. It will be observed that digestion occurred in acid as well as in alkaline media. Similar observations were obtained qualitatively by Mesnil (7) in the case of actinia. He believed the proteolytic enzyme in this coelenterate to be unique in that it was active over a wide range of acidity and alkalinity. He designated this enzyme as "actino-diastase." We believe, however, that our results indicate the presence of two enzymes, a pepsin and a trypsin, the latter predominating. Two optima are

TABLE 1

Digestion of gelatin by physalia

15 cc. gelatin + HCl or NaOH + 2 gm. tissue preparation + water = 30 cc.

TUBE NUMBER	ADDED		INITIAL pH	DEGREE OF LIQUEFACTION AFTER DIGESTION AT 37°C. FOR		
	HCl	NaOH		30 minutes	60 minutes	120 minutes
1	6.0 cc. N		1.7	1	1½	1½
2	4.0 cc. N		2.2	½	1	1
3	3.0 cc. N		2.6	¼	1¾	2
4	1.6 cc. N		3.6	0	1¾	1¾
5	6.0 cc. 0.1 N		4.0	0	0	1½
6			5.8	1½	5¾	6
7		3.0 cc. 0.1 N	7.0	6	6	
8		4.0 cc. 0.1 N	7.3	6	6	
9		6.0 cc. 0.1 N	7.7	5½	6	
10		7.0 cc. 0.1 N	7.8	4½	6	
11		8.0 cc. 0.1 N	8.0	4	6	

apparent from the data presented in tables 1 and 2, the first at a pH of about 3.0, and the second approximating pH 7.3. These optima correspond closely to those obtained by Dernby with commercial pepsin and trypsin preparations. We have confirmed these findings in unreported experiments. The results obtained with the mesenteric filaments of the jellyfish also indicated peptic as well as tryptic digestion (cf. table 2). It appears from a number of our experiments that the mesenteric filaments of animals killed during the height of digestion have greater enzymatic activity than the filaments of animals containing no food in their gastric cavities. Confirmatory evidence has been adduced to show that the proteolytic enzymes of the two coelenterates

closely resemble pepsin and trypsin of higher forms. By using varying proportions of commercial pepsin and trypsin, we have been able to produce effects on gelatin similar to those obtained with enzyme preparations of the jellyfish and the Portuguese-man-of-war.

It next seemed of interest to compare the digestion of various proteins by the proteolytic enzymes of the coelenterates. In the experiments with the siphons of Physalia, the following proteins were tested: egg albumin, coagulated egg albumin, fish meat, beef, fibrin and casein. The digests, preserved with toluene and chloroform, were made in duplicate and were suitably controlled with boiled tissue preparations. At stated intervals, portions were removed and saturated with ammo-

TABLE 2

Digestion of gelatin by stomolophus

15 cc. gelatin + HCl or NaOH + 2 gm. tissue preparation + water = 30 cc.

TUBE NUMBER	ADDED		INITIAL pH	DEGREE OF LIQUEFACTION AFTER DIGESTION AT 37°C. FOR		
	HCl	NaOH		90 minutes	150 minutes	270 minutes
1	5.0 cc. N		1.8	¼	1	1¼
2	3.0 cc. N		2.6	1	1½	1½
3	2.0 cc. N		3.2	1¾	1¾	2
4	1.6 cc. N		3.6	1¾	1¾	2
5	6.0 cc. 0.1 N		4.0	0	1½	2
6			5.6	½	1	1¼
7		3.0 cc. 0.1 N	7.0	1¾	2¾	
8		4.0 cc. 0.1 N	7.3	1½	2½	
9		6.0 cc. 0.1 N	7.7	1½	2¼	
10		7.0 cc. 0.1 N	7.8	1½	2½	
11		8.0 cc. 0.1 N	8.0	1¾	2¼	

nium sulphate. In the tests for trypsin, the filtrates were examined for peptones (biuret) and for tryptophane (bromine water). The results, not reported here in detail, indicated a striking contrast between the rapidity of tryptic digestion of gelatin and the slow rate of digestion of other proteins. Even after a week the amino-acid stage had not been reached in the coagulated egg albumin digests. Of the proteins tested, fish meat appeared to be digested most rapidly. In the pepsin digests, hydrolysis progressed very slowly, the peptone stage not being reached until 72 to 120 hours had intervened. Of the several proteins tested, beef was digested most rapidly. Peptones were detected in the latter case after 24 hours. On the other hand, the coagulated egg albumin

digests had not progressed to the peptone stage until a period of a week had elapsed.

Observations on the digestive action of the mesenteric filaments were also made. Peptic digestion of egg albumin occurred more slowly than that of fish or beef. In the tests for tryptic digestion, peptones were detected in the beef and fish digests after 24 hours had elapsed. Tests for tryptophane remained negative even after 5 days. These results indicate the relative inactivity of the proteolytic enzymes of Stomolophus meleagris as compared with those of higher animals.

The tissue preparations used in all experiments were routinely tested for rennitic action. Both in Physalia arethusa and Stomolophus meleagris, the presence of a milk coagulating enzyme was invariably demonstrated. As a rule, the tissue suspensions of the Portuguese-man-of-war were quite active. Two cubic centimeters, when added to 5 cc. of milk, produced a firm clot in 5 to 10 minutes. With the mesenteric filaments of the jellyfish, clotting occurred more slowly.

Lipolytic enzymes. In view of the slight lipolytic action of the digestive tissues of coelenterates, we made use of soluble esters such as ethyl butyrate and amyl acetate, in most of our experiments. Although these esters have been frequently employed by many investigators in lipolytic studies, their use is not without objection. A distinction has been made by a number of authors, notably Arthus (16), Oppenheimer (17) and Jansen (18), between the esterases—enzymes which hydrolyze lower esters but not neutral fats—and the lipases. In addition to the experiments with ethyl butyrate and amyl acetate, the lipolytic action of the tissue preparations was frequently tested by means of litmus milk. In table 3 are given the results of a number of typical experiments in which ethyl butyrate and amyl acetate were subjected to the action of the mesenteric filaments of the jellyfish and the siphons of the Portuguese-man-of-war. After incubation for 48 hours at 37°C., the degree of hydrolysis of the esters was determined by titration with N/20 NaOH. Our observations clearly indicate the presence of enzymes capable of slowly hydrolyzing ethyl butyrate and amyl acetate. Evidence of fat digestion, as determined by the litmus milk test, was likewise obtained with the tissue suspensions. Indication of digestion in the latter was usually manifested within 10 to 24 hours.

Amylolytic and inverting enzymes. One of the factors which had to be considered in investigating the amylolytic and inverting enzymes, was the possible glycolytic effect of the tissues. If the destruction of

glucose were to occur simultaneously with the inversion of the disaccharides, the effect produced by amylase, maltase, lactase and invertase would be masked to a greater or less degree. A number of tests, performed by subjecting glucose solutions to the action of the digestive tissues, failed to reveal any glucose destruction. In our investigations, the carbohydate digesting enzymes were detected by measuring the increase in reducing power of the substrates as digestion progressed. For this purpose, the Benedict modification (19) of the Lewis-Benedict blood sugar method (20) was found convenient.

Three experiments were performed in studying the diastatic activity of the Portuguese-man-of-war. In the first two, wet tissue preparations, made by macerating the siphons of several individuals, were used. In the third experiment, an enzyme preparation was employed

TABLE 3

Ester hydrolysis

3 cc. of ester + 3 cc. of tissue suspension + 10 cc. of water

ANIMAL	ESTER	N/20 NaOH REQUIRED TO NEUTRALIZE							
		Experiments				Controls			
		I	II	III	Average	I	II	III	Average
Physalia	Ethyl butyrate	6.85	6.30	6.00	6.38	3.55	3.50	3.80	3.62
Physalia	Amyl acetate	5.90	5.80	6.05	5.92	3.55	3.70	3.70	3.65
Stomolophus	Ethyl butyrate	4.20	6.15	5.00	5.12	1.55	1.50	1.30	1.45

which had been made by drying the siphons for several days at 37°C. Definite quantities of the enzyme were added to 15 cc. portions of 1.8 per cent starch solution (equivalent to 2 per cent glucose). Sufficient 0.9 per cent NaCl solution was added to bring the total volume to 30 cc. The initial reducing power of the contents of each flask was determined. After incubation at 37°C., portions of the digests were removed at definite intervals and analyzed. The reducing power is expressed in terms of milligrams of glucose in 1 cc. of the digest. All experiments were controlled with boiled enzyme preparations. As indicated in table 4, an amylolytic enzyme is present in the siphons of Physalia arethusa. For the presence of inulinase, tests were made by incubating siphon suspensions with inulin solutions. Two experiments were performed. As indicated in table 5, no increase in the reducing power of the digests was produced in either case. From this

it is concluded that inulinase does not occur in the siphons of Physalia arethusa.

In testing for maltase, definite quantities of siphon preparations, made in the usual way, were added to 15 cc. portions of 1.9 per cent maltose (equivalent to 2 per cent glucose). Sufficient 0.9 per cent NaCl solution was added to make the total volume 30 cc. The re-

TABLE 4

Amylase

EXPERIMENT NUMBER	AMOUNT OF ENZYME	REDUCING POWER IN MILLIGRAMS OF GLUCOSE IN 1 CC. OF THE DIGEST				REMARKS
		Initial	6 hours	24 hours	48 hours	
	grams					
I	2	0.18		1.28	1.86	Experiment
	2	0.18		0.30	0.33	Control
II	2	0.22		1.42	2.56	Experiment
	2	0.22		0.24	0.31	Control
III	2	0.14	0.96	2.14		Dry preparation
	2	0.14	0.14	0.18		Control

TABLE 5

Inulinase

EXPERIMENT NUMBER	AMOUNT OF ENZYME	REDUCING POWER IN MILLIGRAMS OF GLUCOSE IN 1 CC. OF THE DIGEST				REMARKS
		Initial	24 hours	44 hours	68 hours	
	grams					
I	2	0.84		0.87	0.88	Experiment
	2	0.84		0.86	0.86	Control
II	3	0.57	0.55			Experiment
	3	0.57	0.55			Control

ducing power was determined at the beginning of the experiment, as well as after 24 hours of digestion. Although at times the effect was very slight, we have never failed to demonstrate the presence of maltase in the siphons of Physalia. Similar technique was employed in the tests for lactase. In contrast to the behavior of the siphons toward maltose, lactose was not digested. The inversion of sucrose could not be demonstrated unless large quantities of the tissue preparations

were used. When dilute raffinose solutions were incubated with siphon preparations, slight hydrolysis, as determined by an increase in the reducing power, was often detected. We do not, however, attribute this action to a specific raffinose-splitting enzyme but rather to the action of invertase. As pointed out by Neuberg (21) and Armstrong and Glover (22), invertase converts raffinose into fructose and melibiose.

Essentially the same procedure was employed in the study of amylase and inulinase of the jellyfish as in the case of the Portuguese-man-of-war. The data, recorded in table 6, demonstrate the presence of amylase and the absence of inulinase from the gastric filaments of Stomolophus. The presence of maltase was repeatedly determined. Lactase, on the other hand, was found to be absent. In a number of experiments, invertase could not be detected. Some preparations did exhibit, however, a very slight capacity to invert sucrose. If present

TABLE 6

Amylase and inulinase in stomolophus

EXPERIMENT	REDUCING POWER IN MILLIGRAMS OF GLUCOSE IN 1 CC. OF THE DIGEST			REMARKS
	Initial	24 hours	48 hours	
Amylase	0.22	1.26	1.92	Test
	0.22	0.24	0.30	Control
Inulinase	0.54	0.55		Test
	0.54	0.55		Control

in the jellyfish at all, invertase occurs in minute traces and, as in the Portuguese-man-of-war, it can not be regarded as playing an important part in the digestive process of this species.

SUMMARY

1. In Physalia arethusa and Stomolophus meleagris the following digestive enzymes have been found: pepsin, trypsin, rennin, amylase, maltase and lipase. Invertase is present in negligible quantity, and is apparently of little significance in the digestive processes of these coelenterates. Both inulinase and lactase are absent.

2. Insofar as digestion of gelatin is concerned, the peptic and tryptic enzymes of the two coelenterates resemble the pepsin and trypsin of higher forms of animal life. Pepsin of either origin digests gelatin most rapidly at a pH of 3.0 to 3.5. Likewise, trypsin of either origin

manifests maximum effect in an alkalinity equivalent to pH 7.3 to 7.6. No evidence has been obtained regarding the existence of a peculiar type of enzyme (actino-diastase), such as suggested by Mesnil in the case of actinia. We believe, on the contrary, that the activity of the proteolytic enzymes of coelenterates over a wide range of acidity and alkalinity is due to the presence of at least two enzymes which are comparable to pepsin and trypsin of higher animals.

3. It appears that proteins which naturally serve as food for the jellyfish and the Portuguese-man-of-war are more readily digested by the enzymes of these species, than are proteins foreign to their customary diet. For instance, fish meat is more easily digested than fibrin or egg albumin. This fact suggests the interesting possibility of the enzymes being adapted specifically to the individual proteins upon which they customarily act.

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COMPARATIVE STUDIES OF DIGESTION

II. Digestion in Elasmobranchs and Teleosts[1]

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Much information of theoretical interest can be obtained from comparative studies of the digestive enzymes in the animal series. As yet there is insufficient evidence to show definitely the differences and similarities between the digestive processes in various animal groups. The influence of acids upon the pepsin of fishes deserves especial attention. In his review of the literature, Scheunert (1) states that the proteolytic enzyme in the stomach of fishes is peptic in nature, but that it is not to be identified with the pepsin of mammals. Most investigations on the digestive enzymes in marine animals were made before the necessity for accurate measurements of the hydrogen ion concentration was appreciated. Much of this work is therefore of but little value, and cannot be correlated with the more careful enzyme studies performed in recent times.

In many teleosts, the intestine branches out near its junction with the stomach into long and slender pouches or caeca, into which, according to Owen (2), food does not enter. Krukenberg (3) studied the functions of the pyloric caeca in a large number of species. In some fishes, he states that the appendages enclose only mucus and chyle, and are organs of absorption. He did not regard the caeca of very great physiological importance despite the fact that in some species the caecal secretions contained a trypsin-like ferment. In one form, Thymnus vulgaris, both pepsin and trypsin, and perhaps an amylase were present. According to Blanchard (4), the pyloric caeca secrete a diastatic as well as a proteolytic enzyme. Stirling (5) found that the pyloric appendages of the herring, cod and hake secrete a tryptic

[1] The data in this paper are taken from the thesis presented by Meyer Bodansky for the degree of Master of Arts, University of Texas, 1922.

enzyme. Decker (6), on the other hand, reported that in the hake this enzyme is comparable to pepsin. Bondouy (7) pointed out that the pyloric tubes in fishes are not organs of absorption, as was stated by Krukenberg. On the contrary, he believes that they have an important digestive function, and secrete a fluid containing trypsin and amylopsin but not lipase.

In the present investigation we have compared the digestive action of the pepsin of fishes with the effect produced by commercial pepsin. For this purpose, gelatin was employed as the substrate. In the future we hope to extend these studies to other proteins. A systematic search has been made also for the enzymes of the pyloric caeca of the red snapper (Lutjanus aya).

We first investigated the digestion of gelatin by the pepsin of three species of elasmobranchs, i.e., shark or dogfish (Squalus acanthias), sawfish (Pristis pectinatus), and the torpedo ray (Torpedo galvani); and four species of teleosts, i.e., red snapper (Lutjanus aya), flounder (Paralichthys lethostigma), cowfish (Lactophrys tricornis, and the black grouper (Mycteroperca bonaci). The Dernby method (8), previously used by us in the study of proteolytic digestion in coelenterates (9), was employed in the present investigation. For the purpose of economizing space, the experiment with shark pepsin alone is herein described in detail.

The gastric mucosas of two sharks (Squalus acanthias) were scraped and triturated with toluene and water. The extract (total volume 30 cc.) was filtered, and 1 cc. of the filtrate was used in each of the tests. The results are given in table 1, and indicate that maximum digestion occurred in an acidity of approximately pH = 3.0.

Experiments performed with the extracts prepared from the gastric mucosas of the other elasmobranchs and teleosts gave similar results. As in the shark, maximum peptic digestion of gelatin always occurred about pH 3.0, the hydrogen ion concentration regarded as the optimum for mammalian pepsin.

According to van Herwerden (10), the optimum acidity for peptic digestion of fibrin in the case of Scyllium is between 0.5 and 1.0 per cent hydrochloric acid. We have performed a number of fibrin digestion experiments with pepsin extracted from the gastric mucosa of Squalus and other species. Invariably digestion of fibrin was observed to be more rapid in 0.2 than in 0.5 per cent hydrochloric acid. Coagulated egg albumin was very slowly digested by fish pepsin. That the slow digestion of the latter protein was not due entirely to the relatively

small surface exposed is indicated by the fact that finely divided coagulated egg albumin was not digested with an appreciably greater rapidity. Furthermore, a commercial pepsin solution capable of producing an effect on fish meat similar in extent to that exerted by fish pepsin, digested coagulated egg albumin quite readily.

Sullivan (11) found that neutralized extracts of the mucosa of the stomachic sacs of elasmobranchs did not coagulate milk. Though stating that the rennitic enzyme and its zymogen were absent from his preparations, he admits in a footnote that certain experiments have led him to believe that the zymogen may exist in the mucosa of at least some species, and might be extracted by appropriate methods. Hammersten (12) prepared an active rennin preparation from the pike. We have tested neutralized extracts of the gastric mucosa of several

TABLE 1

Digestion of gelatin by shark pepsin

15 cc. gelatin solution + HCl + 1.0 cc. enzyme + water = 30 cc.

TUBE NUMBER	ADDED HCl	INITIAL pH	DEGREE OF LIQUEFACTION AFTER DIGESTION AT 37°C. FOR		
			60 minutes	180 minutes	300 minutes
1	5.0 cc. N	1.8	1	2½	3
2	3.0 cc. N	2.6	1½	3½	4
3	2.0 cc. N	3.2	1½	3¾	4
4	1.0 cc. N	3.8	¼	1½	2
5	6.0 cc. 0.1 N	4.0	¼	¾	1
6		5.8	0	0	0

species for rennin. The tests were performed in most cases on a number of different individuals and were suitably controlled. Except for variations in the time of clotting, the results for any one species were always uniform. It appears from the data in table 2 that a rennitic enzyme is present in some forms and is absent in others. The stomachs of the 10 red snappers used in test no. 7 were empty; those employed in test no. 8 were at the height of digestion. Enzyme extracts obtained from animals containing food were found to be more active than were extracts secured from animals with empty stomachs.

The digestive function of the pyloric appendages of the red snapper (*Lutjanus aya*). The red snapper possesses five well-developed pyloric caeca. In the preparation of enzyme extracts, the caeca were cut about midway between the blind end and the intestines, the distal

portions only being used. The object of doing this was to prevent, as much as possible, any contamination wth material that may have entered from the alimentary canal.

For the purpose of studying the proteolytic enzymes, a glycerol-water extract, 60 cc. in volume, was prepared from the caecal mucosas of 15 animals. One cubic centimeter of the resulting preparation was used in each of a series of gelatin tests. The data presented in table 3 indicate the presence of a peptic as well as a tryptic enzyme. This was confirmed by a number of digestion experiments in which beef, fish fibrin, casein and coagulated egg albumin were used. In a number of

TABLE 2

Rennin

NUMBER	ANIMAL	NUMBER OF SPECIMENS	TEST	TIME OF CLOTTING
	Elasmobranchii			
				minutes
1	Shark (Squalus acanthias)	2	−	
2	Sawfish (Pristis pectinatus)	1	−	
3	Torpedo ray (Torpedo galvani?)	1	+	7
	Teleostomi			
4	Flounder (Paralichthys lethostigma)	1	+	25
5	Mullet (Mugil cephalus)	5	−	
6	Cowfish (Lactophrys tricornis)	1	−	
7	Red snapper (Lutjanus aya)	10	+	55
8	Red snapper (Lutjanus aya)	3	+	20
9	Catfish (Ailurichthys marinus)	1	+	20

tests with ethyl butyrate and litmus milk, we have detected weak lipolytic action in the extracts of the pyloric appendages of the red snapper (cf. table 4).

The technique described in our study of the amylolytic and inverting enzymes of the coelenterates (9) was applied to the present investigation. The rapidity of starch hydrolysis and the absence of inulinase are indicated by the results presented in table 5. We have been unable to detect the presence of maltase or lactase in the pyloric appendages of the red snapper. Invertase, on the other hand, occurs in minute traces.

TABLE 3

Digestion of gelatin by pyloric caeca

15 cc. gelatin + HCl or NaOH + 1.0 cc. enzyme solution + water = 30 cc.

TUBE NUMBER	ADDED		INITIAL pH	DEGREE OF LIQUEFACTION AFTER DIGESTION AT 37°C. FOR	
	HCl	NaOH		30 minutes	60 minutes
1	6.0 cc. N		1.7	½	1½
2	4.0 cc. N		2.2	½	2
3	3.0 cc. N		2.6	1	2
4	1.6 cc. N		3.6	½	1
5	6.0 cc. 0.1 N		4.0	¾	1¼
6			5.8	1	2
7		3.0 cc. 0.1 N	7.0	2	6
8		4.0 cc. 0.1 N	7.3	4½	6
9		6.0 cc. 0.1 N	7.7	6	6
10		7.0 cc. 0.1 N	7.8	6	6
11		8.0 cc. 0.1 N	8.0	6	6

TABLE 4

Hydrolysis of ethyl butyrate by pyloric caeca

3 cc. of ethyl butyrate + 3 cc. enzyme preparation + 10 cc. of water

NUMBER	N/20 NaOH REQUIRED TO NEUTRALIZE	
	Experiment	Control
	cc.	cc.
1	13.0	5.6
2	13.5	4.9
3	12.3	4.6
Average.....	12.9	5.0

TABLE 5

Amylase

EXPERIMENT NUMBER		REDUCING POWER IN MGM. OF GLUCOSE IN 1 CC.		
		Initial	24 hours	48 hours
I	Test.....	0.26	1.23	1.96
	Control.....	0.26	0.28	0.28
II	Test.....	0.17	0.52	0.96
	Control.....	0.17	0.19	0.22

SUMMARY

1. The optimum hydrogen ion concentration for gelatin digestion by fish pepsin is about pH = 3.0, and corresponds with the optimum for mammalian pepsin. Fibrin is more rapidly digested by the pepsin of elasmobranchs and teleosts in 0.2 than in 0.5 per cent hydrochloric acid.

2. Coagulated egg albumin is very slowly digested by the proteolytic enzymes of fishes. Fish pepsin is capable of digesting effectively a larger variety of proteins than is the pepsin of either the jellyfish or the Portuguese-man-of-war.

3. The stomachic sac of the torpedo ray secretes a rennitic enzyme. In the dogfish and sawfish rennin is absent. Some teleosts (red snapper, flounder, and catfish), possess gastric rennin; others (cowfish and mullet) do not.

4. The pyloric caeca of the red snapper contain trypsin, pepsin, rennin, amylopsin, and lipase (weak). Inulinase, maltase and lactase are absent. Invertase is present to a slight extent. It therefore appears that the secretions of the pyloric appendages of Lutjanus aya contain most of the enzymes which are usually found in the pancreatic juice of higher vertebrates.

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THE INFLUENCE OF ADRENALIN ON METABOLISM IN VARIOUS EXCISED TISSUES

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In a recent paper (1) we reported some experiments showing that the production of carbon dioxid in excised skeletal muscles of the frog is markedly augmented by the addition of adrenalin to the solutions in which the muscles are immersed. We interpreted the findings as supporting the view of Boothby and Sandiford (2) that adrenalin exerts a thermogenic influence upon living tissue. Near the close of that paper we pointed out the necessity of further experimentation in order to determine whether the thermogenic effect of adrenalin is confined to skeletal muscle or applies to other tissues as well.

Between the sending of our paper to press and its appearance in publication, the observations of Garrey (3) appeared, with the finding that the rate of carbon dioxid production is markedly augmented in the cardiac ganglion of Limulus by the addition of adrenalin chloride to the solution in which the ganglion is immersed. This result of Garrey's shows that the thermogenic influence of adrenalin is operative at least in nervous as well as in muscular tissue. We have to report in this contribution experiments showing that this influence extends to still other kinds of tissue.

Our experiments were all performed on excised tissues of the frog. The method was essentially that described in our former paper (1), our only difference in procedure arising from the fact that in the study of non-muscular tissues no precautions needed to be observed to assure ourselves of the absence of contraction. These tissues were, therefore, not attached to recording levers, but were simply lowered into tubes containing the solution and indicator, and stoppered tightly with paraffined corks.

The quantitative expression of acid production. In order to make different experiments readily comparable we adopted in our former paper, and have continued in this, the practice of expressing our results in

terms of acid production per gram of tissue per hour. The computations are made according to the formula:

$$A_c = \frac{60\ C_H}{w\ t}$$

in which A_c is the calculated acid production, w the weight in grams, t the time in minutes, and C_H the difference between the final and initial hydrogen ion concentrations of the solution. For converting pH values into C_H we used a table adapted from McClendon's chart (4).

The use of the above formula involves the assumption that acid production is a linear function both of the weight of the tissue and of the time. This assumption appears to be justified so far as weight of tissue is concerned. It introduces some possibility of error in respect to the correction for time since, as A. R. Moore has shown (5), the rate of acid production in excised tissues is not strictly uniform, but varies hour by hour, at least for the first two or three hours. The error thus introduced is probably not excessive in comparison with other sources of error inherent in the method; thus Moore (loc. cit.) sets the limit of error in making color comparisons at 10 per cent; unavoidable variations in the time between excision and immersion in the test solution, or unknown differences in the metabolic states of the animals from which the test tissues are taken, offer possibilities of equal or even greater percentages of error. On the whole we consider our scheme of expressing our results in terms of acid production per gram of tissue per hour entirely sound from the standpoint of freedom from undue error.

The reduction to a standard temperature (20°C.) was made by the use of Kanitz' formula (6):

$$Q_{10} = \left(\frac{K_2}{K_1}\right)^{\frac{10}{t_2 - t_1}}$$

In applying this formula K_2 is the observed acid production at observed temperature t_2, when t_2 is higher than 20°; and K_1 the computed acid production at 20°, here assigned the designation t_1. For observed temperatures below 20° the significance of K_2 and K_1 is reversed, as also of t_2 and t_1. The precise value to be assigned to Q_{10} is somewhat problematical. Garrey (7) obtained, in one series of investigations, values of Q_{10} of about 2 at temperatures near 20°C. In the absence of specific data relative to the particular tissues used by us we decided to adopt the uniform practice of calling Q_{10}, 2. As a matter of fact, the

use for Q_{10} of the value 2.4, as found in similar studies by some other investigators to be more nearly correct, would change the calculated acid productions for 20° in our series by only about 3 to 5 per cent, showing the relative unimportance of extreme accuracy in deciding upon a value of Q_{10} for the purpose of correcting for temperature when, as in our experiments, the observed extremes are not more than 3 or 4 degrees removed from the standard. We should state that in attempting in our former paper to adapt the Kanitz formula for convenient solution with a slide rule we introduced a systematic error whose effect was to make all the corrected acid productions too high by an amount averaging slightly less than 2 per cent. Thus the average acid production of frogs' excised sartorius muscles, per gram per hour, stated by us to be 110×10^{-8}, becomes, when calculated in exact accordance with the Kanitz formula, 108×10^{-8}. All the corrected figures reported in this paper were computed in the more accurate manner.

Frogs' brains. In preparing a brain for experiment the spinal cord was first pithed in the usual manner; then the roof of the cranium was removed with great care, working forward from the point of pithing. All nerves leading away from the brain were cut across, with care to avoid injury to the brain itself, and the entire organ was then lifted out of the cranial cavity without having been torn or bruised in any degree whatsoever. By means of a sharp razor blade the brain was bisected sagittally, thus giving from a single brain two masses of tissue of substantially equal weight, one for a control, and the other for an experiment with adrenalin. Ten brains were tested in all. The weight of one half-brain ranged from 131 to 159 mgm., averaging 148 mgm. The greatest difference in weight between two halves of the same brain was 3 mgm. Adrenalin (the dry powder dissolved in Ringer's solution—formula for cold-blooded animals) was used in concentration of 1:200,000. The hydrogen ion concentration, expressed as pH, was brought to 8 in both the control and the adrenalin tube at the beginning of each experiment by adding a minute amount of alkali.

The acid productions for the control brains in Ringer's fluid and for the test brains in adrenalin-containing Ringer's solution are set down in detail in table 1. The table shows an average for the controls of 91.5×10^{-8} at 20°C., indicating a resting metabolism not much below that of skeletal muscle, as found by us in our former work (see above, p. 490). The augmentation by adrenalin is very marked, averaging a 2.6-fold increase. This is somewhat greater than the effect noted by

Garrey (2), whose greatest increase, for Limulus ganglion, was two-fold. It should be noted in this connection that Garrey used adrenalin chloride in concentration of 1:10,000, as compared with 1:200,000 adrenalin employed by us.

Mesonephros. Two series of experiments with mesonephric tissue were carried on. In the first six frogs were used, the concentration of adrenalin being 1:100,000; in the second four frogs, and adrenalin in concentration of 1:200,000, were employed. The method of dissecting the mesonephros was as follows: with fine scissors the mesentery was cut through on the sides and the cardinal vein in the mid-line. The

TABLE 1

Thermogenic influence of adrenalin on brain tissue

DATE	TEMPERATURE	ACID PRODUCTION		CALCULATED ACID PRODUCTION AT 20°		RATIO ADRENALIN EXPERIMENTS TO CONTROLS
		Control	Adrenalin 1:200000	Control	Adrenalin 1:200000	
1922	°C.					
February 22	22.0	78.4	226	68.3	196.5	2.88
		89.0	259	77.5	225.5	2.91
		96.5	227	84.0	197.5	2.35
		118.0	290	102.7	252.5	2.45
		95.5	238	83.1	207.0	2.45
		160.0	388	139.3	338.0	2.43
		96.0	241	83.6	209.3	2.51
		131.0	321	114.0	279.5	2.45
		104.5	299	91.0	260.5	2.85
		81.8	226	71.2	196.5	2.76
Averages				91.5	236.3	2.60

Note: All figures for acid production should be multiplied by 10^{-8}.

organ was then lifted clear by means of fine forceps, without appreciable injury to the mesonephric tissue. The average weight of the single organs was 132 mgm.; the range was from 100 to 210 mgm.; the greatest difference between the members of a pair was 20 mgm.; the average difference, 6 mgm. The initial pH, of both control and test solutions, was either 7.9 or 8.0. The results of the experiments are given in table 2. The acid production in the controls averages 17×10^{-8} per gram per hour at 20°C., a markedly lower figure than found in either skeletal muscle or nerve tissue. The augmentation by adrenalin averages a 3-fold increase, and is fully as marked in the 1:200,000 series as in that in which 1:100,000 adrenalin was used.

Liver. Small pieces of liver tissue were cut with no more injury than necessary and then bisected by means of a sharp razor blade. The average weight of single test pieces was 450 mgm.; the range was from 360 to 510 mgm.; the average difference between members of a pair was 26 mgm. The results of the ten experiments are given in table 3. The acid production in the controls averages only 6.5 $\times$ 10^{-8} per gram per hour at 20°C., by far the lowest level of metabolism encountered by us in our experiments, and of particular interest in view of the fact that tissue injury was necessarily greater in the preparation of these test tissues than in any others of our series. The aug-

TABLE 2

Thermogenic influence of adrenalin on mesonephric tissue

DATE	TEMPERATURE	ACID PRODUCTION		CALCULATED ACID PRODUCTION AT 20°		RATIO ADRENALIN EXPERIMENTS TO CONTROLS
		Control	Adrenalin 1:100000	Control	Adrenalin	
1922	°C.					
February 3	20.0			8.6	25.8	3.00
				18.3	37.0	2.04
				23.3	52.0	2.23
				11.3	47.1	4.16
			1:200000	16.6	45.4	2.73
				36.1	85.6	2.37
February 10	18.5	18.5	71.0	20.5	78.7	3.84
		10.9	37.0	12.1	41.1	3.40
		11.4	58.0	12.6	64.5	5.12
		10.3	28.8	11.4	32.0	2.81
Averages				17.1	50.1	3.17

mentation by adrenalin averages a 2.5-fold increase; the concentration of adrenalin was 1:200,000 throughout.

Stomach. To avoid excessive injury to tissue we made no attempt to separate mucous and muscular layers for the purpose of testing each individually, but merely dissected out the stomach and bisected it, thus obtaining from a single frog both a control and an adrenalin portion. The average weight of a single test piece was 325 mgm.; the range was from 307 to 347 mgm.; the greatest difference between members of a pair was 7 mgm. The results of the five experiments are give in table 4. The acid production in the controls averages 141 $\times$ 10^{-8} per gram per hour at 20°C. This figure, which is higher by 30 per cent

than we have observed in any other tissue, and nearly double that given by a closely similar tissue, intestinal, results from which are given in the next paragraph, seems to us to indicate the strong probability that the presence of the gastric glands has something to do with the

TABLE 3

Thermogenic influence of adrenalin on liver tissue

DATE	TEMPERATURE	ACID PRODUCTION		CALCULATED ACID PRODUCTION AT 20°		RATIO ADRENALIN EXPERIMENTS TO CONTROLS
		Control	Adrenalin 1:200000	Control	Adrenalin 1:200000	
1922	°C.					
February 10	18.5	6.25	16.4	7.00	18.2	2.60
		7.49	13.5	8.30	15.0	1.81
		9.22	22.8	10.30	25.4	2.47
		9.59	15.5	10.60	17.2	1.62
February 22	22.0	6.82	16.6	5.94	14.4	2.43
		6.36	15.6	5.54	13.5	2.44
		4.26	11.6	3.71	10.1	2.72
		6.36	20.5	5.54	17.9	3.23
		4.25	11.2	3.70	9.8	2.65
		4.65	16.2	4.04	14.1	3.49
Averages				6.47	16.0	2.55

TABLE 4

Thermogenic influence of adrenalin on stomach tissue

DATE	TEMPERATURE	ACID PRODUCTION		CALCULATED ACID PRODUCTION AT 20°		RATIO ADRENALIN EXPERIMENTS TO CONTROLS
		Control	Adrenalin 1:200000	Control	Adrenalin 1:200000	
1922	°C.					
March 1	22.0	174	228	151.5	198.5	1.31
		163	312	142.0	271.4	1.91
		160	288	139.3	252.0	1.81
		141	200	122.0	174.0	1.43
		170	270	148.0	235.0	1.59
Averages				141.0	226.2	1.61

large yield of acid. The augmentation by adrenalin averages a 1.6-fold increase; the concentration of adrenalin used was 1:200,000.

Intestine. This tissue was prepared by simply dissecting out a length of small intestine and dividing it as accurately as possible into

two equal portions. The average weight of a single test piece was 282 mgm.; the range was from 268 to 294 mgm.; the greatest difference between the members of a pair was 6 mgm. The results of the five experiments are given in table 5. The acid production in the controls averages 78.5 $\times$ 10^{-8} per gram per hour at 20°C. The augmentation by adrenalin averages only a 1.33-fold increase, which is the smallest we have observed in any tissue under the influence of 1:200,000 adrenalin, which is the concentration used.

Discussion. By means of these experiments we have shown that in a representative series of tissues, including most of the important kinds, there is marked augmentation of metabolism when adrenalin is added to the Ringer's solution in which the tissue is immersed. It

TABLE 5

Thermogenic influence of adrenalin on intestinal tissue

DATE	TEMPERATURE	ACID PRODUCTION		CALCULATED ACID PRODUCTION AT 20°		RATIO ADRENALIN EXPERIMENTS TO CONTROLS
		Control	Adrenalin 1:200000	Control	Adrenalln 1:200000	
1922	°C.					
March 4	20.0			73.5	96.0	1.31
				91.7	124.5	1.36
				58.5	78.4	1.34
				93.9	128.3	1.37
				74.5	95.5	1.28
Averages				78.5	104.5	1.33

is perhaps significant that in all tissues examined, with the exception of those of the alimentary tract, the extent of augmentation was such as to give a resultant total metabolism amounting to about two and one-half times the normal. Reference to the table on page 41 of our former paper (1) shows that in skeletal muscle, under dosages of adrenalin comparable to those used in these experiments, the augmentation was of a similar order of magnitude. The circumstance that in tissues from the alimentary tract the augmentation was much less marked may be without special meaning, but we would call attention to the fact that in these structures, and particularly in the small intestine, where our least augmentation was seen, there exists a mechanism whereby adrenalin may exercise an inhibitory influence. It is conceivable that

this influence may have been operative through the nerve terminals in such a manner as to counteract in part the augmentation of metabolism through the direct action of the hormone on the tissue cells.

SUMMARY

1. Frogs' brains, excised from the body and immersed in Ringer's fluid, showed an acid production averaging, per gram per hour, 91.5 $\times 10^{-8}$, expressed as C_H at 20°C. The addition of adrenalin in concentration of 1:200,000 resulted in an average 2.6-fold augmentation.

2. Frogs' mesonephric tissue, under similar circumstances, gave an average acid production of 17 $\times 10^{-8}$. The addition of adrenalin in concentration of 1:100,000 or 1:200,000 resulted in an average 3-fold augmentation.

3. Frogs' liver tissue, also under similar circumstances, gave an average acid production of 6.5 $\times 10^{-8}$. The addition of adrenalin in concentration of 1:200,000 resulted in an average 2.5-fold augmentation.

4. Frogs' stomach tissue, including both muscular and mucous coats, gave an average acid production of 141 $\times 10^{-8}$. The addition of adrenalin in concentration of 1:200,000 resulted in an average 1.6-fold augmentation.

5. Frogs' intestinal tissue, also including both muscular and mucous coats, gave an average acid production of 78.5 $\times 10^{-8}$. The addition of adrenalin in concentration of 1:200,000 resulted in a 1.33-fold augmentation.

6. These experiments are interpreted as showing that the thermogenic influence of adrenalin is not specific for any one kind of tissue but extends to most sorts, if not to all.

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A STUDY OF THE ACTION CURRENTS OF NERVE WITH THE CATHODE RAY OSCILLOGRAPH

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The instruments which have been used heretofore for the observation of nerve action currents have been galvanometers of the moving coil or magnet type with or without the aid of a rheotome, the capillary electrometer and the string galvanometer. Each of these instruments possesses considerable inertia. The slower galvanometers are unaffected by single action currents and give a sustained deflection when the action currents are monophasic and rapidly repeated. The size of the deflection is the mean value of the individual waves which in turn depends upon their shape and the rate at which they are repeated. As the former is not known the deflections are mainly of qualitative significance.

All of these instruments are governed by the laws of forced vibration with damping. The differential equation expressing this law is

$$M\frac{d_2y}{dt^2} + D\frac{dy}{dt} + Cy = f(t) \qquad (1)$$

which says that the applied force, $f(t)$, produces a motion which is determined by the mass, M, the damping, D, and another restoring force, C, the mass effect being greater when the acceleration, $\frac{d_2y}{dt^2}$, is large; the damping increasing with the velocity, $\frac{dy}{dt}$; and the restoring force, C, increasing with the deviation of the system from equilibrium.

The string galvanometer record gives the most nearly correct picture of the action current. Unfortunately, no sufficiently accurate means are available for correcting the curve to its true form. Valuable papers on the physics of the string galvanometer have been written by Einthoven (1) and by Fahr (2). The latter is more helpful in calculating the form in which a proven function will be returned by the string than in

determining the form of the original disturbance from the photographed record.

It is obvious that the curve given by the string is the graph of the curve expressing the value of y which is the solution of the above equation. To get the true form, i.e., $f(t)$, therefore, one would have to operate on y as indicated in the first member of the above equation. Such an attempt has been made by one of us (H. S. G.). While the operation is simple in theory it presents great practical difficulties. The method of determination depends upon a comparison of the mathematical solution of the movement of the string when a constant current is applied with the curve actually produced by the string. C can be readily determined. If M and D are determined from the curve produced by a very sensitive string as suggested by Fahr (p. 110), D may be determined, but M being found in a small remainder out in the less significant figures cannot be determined with any accuracy. M and D may also be calculated, when the frequencies of oscillation of two tightly stretched strings of known tension and therefore of known values of C have been determined, from the fact that the period of oscillation equals $\frac{4\pi M}{\sqrt{4CM-D^2}}$. The values found by this method are in fair accord with those obtained by the former method and also with those obtained by the method of the modulus of decay. In the actual correction of an action current curve the principal difficulty lies in the determination of the acceleration with satisfactory accuracy, and if one attempts to correct such a curve one finds that the first or acceleration term plays a large part in the correction. It is this term which is responsible for the initial upward concavity of the string record of a constant current. In the case of the action current of nerve the quick portion is completed in less than 2σ, which is during the period in which the string is actually within this initial concave portion. The accuracy of the corrected curve is dependent upon the accuracy with which the rate of change of direction of this portion of the curve can be measured. How great this correction is can be seen in a comparison of the record from a string galvanometer with the true form and potential as determined immediately afterwards, by means of the Braun tube oscillograph. The record (fig. 1) was made from the sciatic nerve of the bull frog using one panel of the amplifier for the string galvanometer and three panels for the Braun tube. Figure 2 is the replica of a Braun tube tracing from the same nerve whose action current is reproduced in figure 7. The records of the action currents as made by these two means are plotted on the same system of coördinates in figure 3. The potentials

for the string galvanometer reproduction are calculated from the calibration of the string with a constant current. It is seen that the greatest distortion by the string is of potentials of very short duration. In the oscillograph record the shock (or "escape"), A, is a distinct curve and the spot returns to the base line before the action current starts. In the string galvanometer reproduction the "escape", a, is still at its crest when the action current starts, and is very much reduced in amplitude.

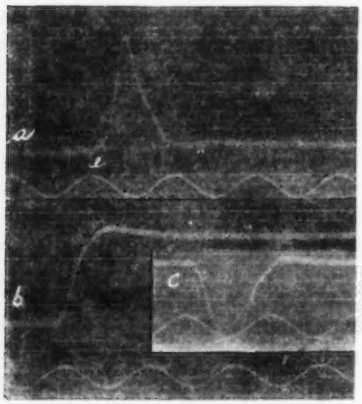

Fig. 1

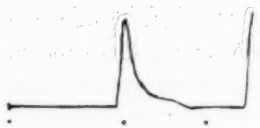

Fig. 2

Fig. 1. String galvanometer records. One double vibration of the tuning fork = 10 σ. a, Action current of the sciatic nerve of the bull frog taken from the same nerve as figures 2 and 8. The short initial wave, e, is due to the shock. b, Calibration of the string and amplifier with a constant current of 3.75 mv. c, String deflection produced by a constant potential of 3.75 mv. lasting about 4.6 σ. In figure 8 is a Braun tube record of a 15 mv. potential of the same duration taken for comparison. (Reduced one-third.)

Fig. 2. Braun tube record of the shock and the beginning of the action current of the bull frog sciatic. X = 6.05 cm.; 1 mf.; 3000 $^{\omega}$ (1 $^{\omega}$ = 1 ohm); dots one sigma apart. (Natural size.)

Similarly it should be noted that it is the quick portion of the action current which is most reduced. In figure 3, C and c, are also reproduced, from figures 1 and 7, the calibration curves of the two instruments. The galvanometer string was so tense as to over-shoot slightly at the application of a constant current. Even so it did not reach its position of second equilibrium for about 4σ (1σ = 0.001 second). The gradual fall in the curve after this point is the effect of the amplifier condensers. The

curve is concave upward for most of the first two sigmas, during which period the most rapid changes in the nerve action current take place, and for this reason correction of string galvanometer records by the laws of the capillary electrometer is unjustifiable. On the other hand, a constant current produces an almost instantaneous rise of the oscillograph spot to its full height.

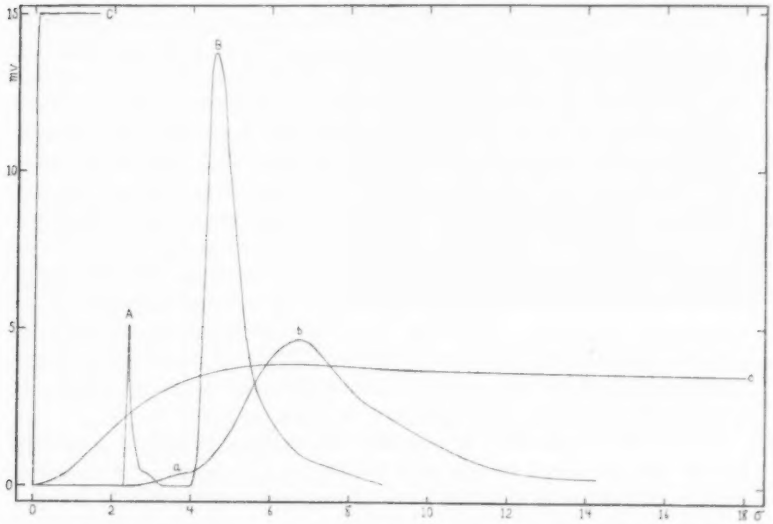

Fig. 3. The action currents of the bull frog sciatic, as recorded by the Braun tube and string galvanometer, plotted in rectangular linear coördinates. *A*, *B*, *C*, Braun tube records; *a*, *b*, *c*, string galvanometer records. *A*, *a*, shock; *B*, *b*, action current; *C*, calibration with a constant current of 15 mv.; *c*, with one of 3.75 mv.

On account of the tedious procedure and the accumulated error in all the measurements involved in the correction of the string galvanometer, the study was temporarily abandoned for the investigation of a less cumbersome method.

While the capillary electrometer shadow is much farther from the true form than the string galvanometer shadow, it can be very much more readily corrected. This is due to the fact that when the electrometer is properly made the effect of the first or acceleration term becomes negligible and therefore the characteristic is the well-known logarithmic

curve without any initial upward concavity in the record produced by a constant current, thus making possible the simple correction as developed by Lippmann, Hermann, Burch, Einthoven and others. The best of the corrected nerve action currents have been made with this instrument.

THE CATHODE RAY OSCILLOGRAPH. The need has long been felt of an inertialess system for recording physiological currents. One would naturally think of the Braun tube in this connection, but heretofore the discrepancy between nerve potentials and the potentials necessary to deflect the cathode stream has been too great to make its use possible. In the older tubes in which the electrons were liberated from the cathode by means of a high potential, e.g., 20,000 to 30,000 volts acting between the cathode and anode, both in a vacuum, the electrons once liberated were given such an acceleration in the potential drop that large forces were necessary for their deflection. With the invention of the hot cathode and Wehnelt cathode the electron emission could be obtained much more easily; but these tubes still operated under a high anode potential to give the electron stream sufficient velocity so that the pencil of rays would not spread as the result of the mutual repulsion of its constituent electrons. In order to avoid the necessity of the high potential and velocity Johnson (3) devised a tube which contains a very small amount of gas and operates upon a 300-volt anode potential. It is this tube which has made the present work possible.[1]

The Johnson tube consists of a Wehnelt cathode heated by a current from a storage battery controlled to about 1.2 amperes by a rheostat. It is by this rheostat that the focus of the spot is controlled. About 1 mm. from the cathode is the anode which is a platinum tube (*A*, fig. 5). Between the anode and cathode there is a 300-volt potential supplied by "Eveready" dry cells. On account of the small amount of gas in the tube, to prevent arcing the anode and cathode are enclosed in a small inside tube. They are separated by a platinum diaphragm which prevents the positive ions formed by electronic bombardment of the gas particles from all impinging on the cathode, thereby overheating and destroying it.

The electron stream passes through the platinum anode, forming a pencil of rays which impinge upon a fluorescent screen of willemite, painted on the end of the tube, and produce a greenish spot. This spot is clearly visible to the dark-adapted eye but has a low actinic value and

[1] We wish to thank Mr. J. B. Johnson of the Western Electric Company for valuable suggestions as to the operation of the Braun tube, and Mr. E. B. Craft of the same Company for supplying us with a tube.

is therefore not easily photographed. Contact prints on photographic films can be made in 5 to 10 seconds, depending upon the rate at which the spot is moving (fig. 4). The easiest way of making permanent records is to trace the standing wave on the end of the tube and then to transfer the record from the tube by means of transparent architect's paper.

As a means of deflecting the stream of rays two pairs of small nickel plates are inserted in the tube. When these plates are connected to a source of potential the electrons move toward the positive member of the pair and away from the negative. If there is a changing potential on only one of the pairs of plates the spot will move along a straight line perpendicular to the pair of plates but if at the same time there is also a potential change on the other pair of plates then the spot will have a motion along the two axes of rectangular coördinates and will describe a figure determined by the nature of the two potential functions. If the motions in the two directions are cyclic and synchronous a standing wave results. Unless the internal deflecting plates are connected conductively to the anode they accumulate a charge and the spot tends to drift. A leak has to be provided, therefore, between the pairs of plates as indicated by L, figure 5.

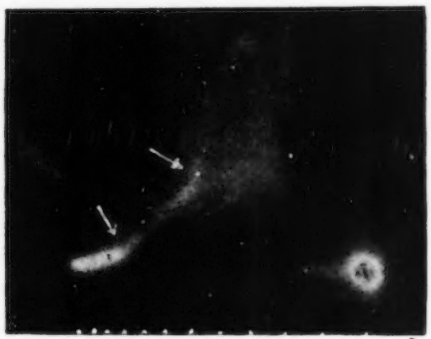

Fig. 4. Bull frog sciatic. The action current was recorded as a contact print on photographic film. To aid the photography the nerve was cooled from 26° (aquarium tank) to 15.4° and a slow deflection of the spot along the abscissa used. $X = 6.05$ cm.; 1 mf.; 9000 ω; distance between dots 1 σ. Time to maximum 1.52 σ. Arrows point to first and second catacrotic notches. On account of the light from the filament, the diffuse fluorescence and the dispersion of the light from the figure, this method is not very satisfactory and gives a poor idea of the actual clearness. (Natural size.)

The electron stream can also be moved by a magnetic field in which case it moves at right angles to the field. Advantage was taken of this fact in placing the zero positionof the spot. Naturally the spot is at rest in about the center of the screen, its exact position depending upon its relation to the earth's field. As it is desirable to start the spot at the left of center as one faces the tube, a magnetic field is

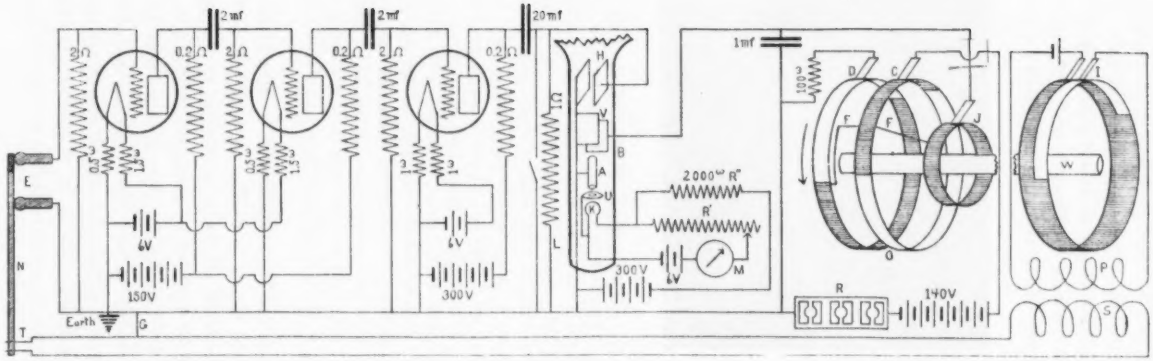

Fig. 5. Diagram of Braun tube oscillograph arranged for observation of nerve action currents. N = nerve, killed end shaded. E = non-polarizable electrodes connected through the three-stage amplifier with the horizontal plates, H, of the Braun tube, B. K = Wehnelt cathode; A = tubular anode; U = diaphragm; L = leak on horizontal plates; M = ammeter; R' = regulating resistance in filament circuit, K; R'' = resistance in anode circuit. The vertical plates, V, connect with the spreading device, O. D = discharging wheel, C = charging wheel and J = continuous contact wheel (metallic parts shaded) all connected conductively by F; R = charging resistance to 1 mf. condenser. I = rotating interrupter in primary circuit, P; S = secondary coil; T = stimulating electrodes grounded through G. 1 Ω = 1 megohm. For the intertube leaks labeled 2 Ω, 0.5 Ω was used.

produced by means of two series-connected solenoids placed one on either side of the neck of the tube. These are so placed that the field is vertical and downward, causing deflection to the left. The circuit consists of a single dry cell, the solenoid, a commutator and a small rheostat to control the strength of the field and therefore the degree of deflection.

The nerve potentials were impressed upon the horizontal or ordinate plates thus producing a vertical movement of the spot. In order to produce a movement along the axis of abscissae a device had to be made which would impress a changing potential on the vertical plates properly synchronized with that on the ordinate plates and also serve to record the time.

THE SPREADING DEVICE. It takes about 23 volts to produce 1 cm. of deflection along the abscissa, therefore 140 to 150 volts are necessary to produce the desired total deflection. Our earliest device, a diagram of which was published in the Proceedings of the American Physiological Society (4), was a rotating potentiometer. A circular resistance was made in segments to each one of which was attached a loop of chromel wire. Across the resistance was a 140-volt drop. One end of the resistance was connected to one of the vertical plates, the other plate was connected to a rotating brush operating on the shaft of a motor with a maximum velocity of 1800 r.p.m. Since the tangential velocity is constant the movement of the spot was linear. As the brush moved along the circular resistance the spot moved linearly to the right; as the brush jumped the gap between the ends of the resistance it came back to zero practically instantaneously, the process being repeated 30 times per second. This spreading device had the advantage that the time was a linear function of the distance along the X axis. It had the disadvantage that the velocity of the spot was determined by the speed of the motor, which therefore had to be recorded. The resistance was easily worn out by the rapid movement of the brush and the speed was limited. At 30 r.p.s. the movement of the spot was 2 M.p.s.

After considerable experimentation with various devices it was found that the most satisfactory scheme was one in which the movement of the spot was controlled by the charge of a condenser. This device has the great advantage that the velocity of the spot is independent of the speed of the motor being dependent only upon the capacity of the condenser and the resistance through which it receives its charge. The condenser being constant the speed is easily varied by changing the resistance. The apparatus has the following construction: a motor is mounted on a

heavy iron base and connected to a shaft in such a way that the shaft may be removed for the setting in place of contact wheels as desired. The motor speed is controlled through a rheostat.

If the inductive reactance is negligible, when a constant potential is impressed across the terminals of a condenser whose initial charge is zero, the potential of the condenser plates at any instant is determined by the expression

$$e - e\,\epsilon^{-\frac{t}{RC}} \tag{2}$$

where e is the impressed potential, ϵ, the base of the natural system of logarithms, t, the time in seconds, R, the resistance in ohms, and C, the capacity in farads. From this expression the desired resistance and capacity may be calculated for the necessary impressed potential of 140 volts. Such a calculation showed that ordinarily a 2-microfarad condenser charged through 1000 to 2000 ohms would give the proper rates of deflection.

The alternate charge and discharge of the condenser is effected by a rotating commutator mounted on the motor shaft. The wheels are made of hard rubber with conducting segments of phosphor-bronze inserted into the periphery. The exact construction can be seen by reference to this portion of the diagram in figure 5. The two large wheels are screwed together and their metallic parts are connected to each other and to the small continuous metallic wheel, J. About one-third of the charging wheel, C, is metallic and about two-thirds of the discharging wheel, D. The conducting segments are so placed that the brushes operating on the periphery are both in contact with the non-conducting rubber for a short segment between the charge and discharge. During the period in which the wheel, C, is making contact, current is flowing into the condenser at a rate determined by the 140-volt battery and the size of the resistance. The plates in the Braun tube are directly connected to the condenser plates and therefore have the same potential as the latter. The spot is thereby deflected at a rate determined by the second term in expression (2), the velocity being greatest at first and then falling off logarithmically. In recording potential changes which are also most rapid at the start, such as the action currents of nerve, this is an advantage. As the commutator wheel rotates, the charging is stopped when the brush runs over onto the rubber, then after a very brief interval the condenser plates are connected through the discharge wheel, D. To prevent sparking as the result of a too rapid discharge, a resistance of 100 ohms is inserted in this circuit; this still allows a

return of the spot to its zero position so rapidly as not to be visible. The process is repeated 20 times per second at the usual motor speed used. The condenser used in the earlier experiments was a 2-mf. paper condenser made by the Western Electric Company for telephone service. The capacity of this condenser was calibrated as follows: The charging time was determined from the rate of rotation of the motor and the length of the conducting segment of the charging wheel. Known high resistances or small intervals were employed which would not allow complete charging and therefore shortened the deflection; then knowing the total potential and the potential attained in the interval, the value of C in expression (2) can be calculated. For the value of the total potential the deflection of the spot when the condenser is fully charged was employed. This involves the assumption that the deflection is a linear function of the impressed potential which practically holds for the amounts of deflection involved.

It then follows from (2) that

$$C = \frac{t}{R \log_\epsilon \frac{X}{X - x}} \tag{3}$$

where X is the total length of the line when the condenser is fully charged, and x is the length of the line for the chosen values of t and R. After proving the accuracy of the method by obtaining in separate determina-

TABLE 1

RESISTANCE	X	x	CALCULATED CAPACITY
ohms			*microfarads*
7,000	5.78	3.7	22.08
10,000	5.65	2.9	21.82
20,000	5.65	1.7	22.08
30,000	5.65	1.2	22.10

tions with different values of R, values of 1.003, 0.995, 0.996 and 1.035 mf. for a 1-mf. condenser whose accuracy is guaranteed by the maker to be within $\frac{1}{4}$ per cent, the condenser used was calibrated. The results are given in table 1. They show that the capacity is a constant when calculated with the aid of different resistances, as is demanded by the theory; therefore when the capacity is known, the value of the time at any point on the abscissa may be obtained.

To render the calculation simple no leak is inserted between the vertical plates of the Braun tube. Such a leak is not necessary as any

accumulation of potential on these plates can pass off through the commutator wheel and the spot has no tendency to drift in the horizontal direction when this is running. In calculating the capacity of the condenser it is necessary to determine the internal resistance of the source of potential as this is added to the known resistance in delaying the filling of the condenser. This value was found to be about 50 ohms. The correction could have been obviated by using the condenser discharge but unfortunately this was not desirable because the spot loses its preciseness of contour when maximally deviated and potential changes occurring on the ordinate plates at the beginning of the discharge of the condenser, when the movement of the spot is fastest, do not produce as clear a figure. As any change in resistance in any part of the condenser circuit particularly at the brush contacts would introduce an error into the time values, the value of C is checked up as a matter of routine at each experiment. In later experiments a precision one microfarad mica condenser was used.

THE AMPLIFIER. As a potential of about 26 volts on the ordinate plates is necessary for a deflection of 1 cm., nerve potentials which lie in the range from 5 to 25 millivolts must be considerably amplified to produce a satisfactory deflection. The amplifier in its present form is shown as a skeleton diagram in figure 5. It was built to embody some improvements suggested by the experiments with the apparatus built by Newcomer and employed by Gasser and Newcomer (5) in a previous investigation. The panels are mounted in separate compartments in a heavy steel box $106 \times 34 \times 34$ cm. in dimension. This rests on a thick cotton mattress and is surrounded by the same material. Each steel compartment is lined with cotton mattresses. The apparatus of each panel is mounted on steel plates about 1 cm. in thickness which rest on rubber sponges lying on the mattress on the floor of the compartment. The plates are grounded to the wall of the box and to them are connected the grid leaks and the negative sides of the filaments. The vacuum tubes are mounted on spongy rubber, so that as the combined result of the damping devices vibration is effectively eliminated. All wires are as short as possible. The connections to the A and B batteries pass directly downward through glass conduits to the battery compartment placed underneath the amplifier proper. Switches are so inserted in each panel that the amplifier may be used as a single panel, a two- or three-tube cascade or as two amplifiers of one tube each, or of one and two tubes. Some changes have been made since the diagram previously

published (4). One *A* battery supplies the first two panels since separate batteries were found to be unnecessary. The third panel has been modified so as to make the dynamic characteristic more nearly linear. The tubes used are designed with an amplification constant of 28 and for a maximum input voltage of two volts. When potentials of the order of those in nerve are applied to the input of the first tube the input potential to the third tube is higher than that for which the tube is designed and in the former system of connection the characteristic deviated from a straight line at input potentials to the amplifier of -5 and $+10$ mv. As no other type of vacuum tube, designed for a high maximum input voltage and having at the same time a high amplification constant, is available it was necessary to increase the negativity of the filament and then to increase the voltage of the plate battery to 300 volts. Accordingly a 3-volt battery was placed in the grid circuit. The negative side of the filament is given a potential of $+1.3$ volts by the resistances in series with it and thus the grid is given a total bias of -4.3 volts.

The Braun tube operates by the potential on its plates and its internal resistance is practically infinite so that it is a very much more favorable instrument for the amplifier to work into than is the string galvanometer. To keep the output resistance as high as possible the leak between the ordinate plates is a small one of 1 megohm. This not only allows a high voltage amplification in the last panel but helps to keep the dynamic characteristic straight.

The voltage amplification of each panel is about 20, making a total between 7,000 and 8,000 depending upon the time the tubes have been in use. The amplification was determined as follows. A dry cell of 1.502 volts was inserted in a circuit interrupted by a tuning fork one hundred times per second, the interruption being necessary on account of the condensers. Connected in this circuit through a commutator was a potentiometer which consisted of a 100,000-ohm resistance box, fractions of which could be led to the input. The tuning fork was adjusted so that the contact period was equal to the open circuit period. This was shown by the fact that the deflection of the beam in the Braun tube appeared as two equally bright spots at the ends of the line. The contact period was therefore 5σ and of sufficient duration to eliminate any effect on the values by the capacity of the resistance box. The deflections in the tube were then compared with the deflection which was produced by an unamplified potential similarly applied to the output condenser, the latter being identical with the deflection produced by the same potential applied to the tube directly. The results of the

calibration showed that a 1 cm. deflection of the spot was produced by 26.4 volts applied directly; by 1.43 volts when amplified by one tube; 0.079 v. by two tubes and 0.00357 v. by three tubes. This means amplifications of about 18.4, 334 and 7400 respectively for one-, two- and three-stage amplifiers, the amplification in the second and third panels being, therefore, 18.1 and 22.1.

For potentials up to 15 millivolts it is convenient to connect the nerve directly to the input. It frequently happens, however, that the nerve develops a potential considerably higher than this, causing the spot to go beyond the limits of the screen. It is also desirable for other reasons to keep the deflection small as corrections are necessary for large deflections. The amplification is decreased too much by the elimination of the third panel of the amplifier, but the desired deflection can be obtained by fractioning the input potential. When this was done by setting up a potentiometer with the 100,000-ohm resistance box used for determining the amplification, the potentials calculated from the fractioned wave regularly proved to be less than those from the unfractioned. This was finally traced to the resistance box. The latter is wound bifilar and therefore presumably has little inductance but an appreciable capacity, and the effect of the latter is to decrease the height of the waves. When the resistance box was replaced by a non-reacting resistance the potentials obtained by calculation from the fractioned waves agreed with those obtained from the direct input. A lavite resistance of 47,500 ohms made by the Western Electric Company is used. It is mounted as a shunt across the input (R', fig. 6) and can be put into use by closing a key.

As the internal resistance of the nerve is by no means negligible with respect to that of the 47,500-ohm shunt, it must be measured. This is done by means of a Kohlrausch bridge made with a non-reacting resistance of two 47,500-ohm units which used either singly or together give the proper resistance in the known arm for the range of nerve resistances. The source of current is a microphone hummer giving 1000 double vibrations per second and a balance is obtained with an appropriate double telephone receiver. The bridge is permanently set up (fig. 6) with a lead easily attached to the nerve connection through a double-pole switch, S', so that determination of the resistance of the nerve and electrodes is only a matter of a few seconds. The potential, E_n, in millivolts at any point on the action current curve can then be calculated from the formula

$$E_n = yV \frac{R_n + 47500}{47500} \qquad (4)$$

where y is the ordinate, V is the number of millivolts per centimeter of deflection, and R_n is the resistance of the nerve. In these determinations the only grid leak from the first tube is through the potentiometer.

The dynamic characteristic of the amplifier working into the Braun tube was determined using the same input potentiometer arrangement as was used for determining the amplification. The results are plotted in

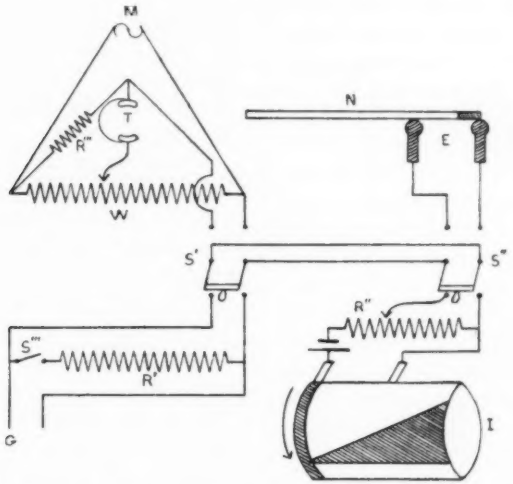

Fig. 6. Diagram of the arrangement of the input apparatus. The nerve, N, is connected directly by the electrodes, E, to the amplifier input, G, by closing the switches, S'' and S', toward the nerve and amplifier and opening the switch, S'''. When S''' is closed the nerve is shunted by R'. Calibration is effected by throwing switch, S'', toward the potentiometer circuit consisting of the resistance, R'', the contact maker, I, and a dry cell. By throwing S'' and S' toward the nerve and the Kohlrausch bridge, the resistance of the former may be measured. M = microphone hummer; R''' = non-reacting resistance; W = slide wire and T = telephone.

figure 7. The linear portion of the characteristic extends from -15 mv. to $+25$ mv. In this experiment the A battery of the first two amplifier panels had been in use for some time and the amplification was 7460. In another determination in which the amplification was 8320 the linear portion of the characteristic was found to be somewhat shorter, especially for negative input potentials. In each instance the characteristic is linear for a longer range of potentials when the input is positive

than when it is negative. The input to the grid of the tube in the third panel has the same sign as the input to the first panel. The grid of the third panel is therefore set farther from the bend produced by saturation and by absorption of current by the grid circuit, due to grid positivity, than it is from the bend due to high internal resistance, as the results of grid negativity. In accord with this finding the nerve preparations are always connected with the cut end to the grid and with the side to the ground. The potential range is from 0 to +15 mv. and therefore on the best portion of the characteristic.

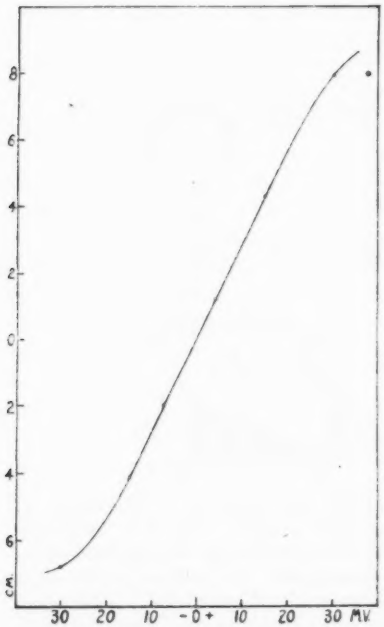

Fig. 7. Dynamic characteristic of the 3-stage amplifier and Braun tube. Abscissa: input in millivolts; ordinate: deflection of spot in centimeters.

The effect of the reactance of the coupling condensers is the same as previously described (5). The effect of the reactance of the output condenser is much less due to the high output resistance. The capacity of the output condenser is 20 mf., the resistance in series with it is the 1-megohm leak on the ordinate plates. In such a circuit the impedance angle is less than 1° for frequencies of 0.5 (per second) and higher, or if a constant potential is impressed on the condenser it takes 0.2 second for a 1 per cent decrease in the potential difference between its plates. Corresponding figures for the intertube condenser circuits are f = 10, and 0.01 second. It can therefore be seen that the condensers are without effect on the form of the functions for which the circuit is designed and the ease of manipulation gives a great advantage over non-reacting circuits.

The amplifier is built as free from inductance as possible by means of short straight wires but any circuit has some inductance, and this will tend to delay the jump in potential at the moment the circuit is made.

Expression (2) does not hold for very small values of t, otherwise the current would jump instantly to its full value which would necessitate the impossible condition of a non-reacting circuit. When a current is impressed upon an electric circuit a finite time elapses during which energy is stored in the electric field. In the original arrangement of the apparatus the amplifier was placed at some distance from the spreader and oscillograph to protect its input from their influence. This involved long connecting wires and the delay in the rise of the calibration curve was longer than that shown in figure 8, due mainly to their inductance. To eliminate this effect the elements of the apparatus were brought closer together and the decreased separation was compensated by better shielding. In the calibration by a constant potential (fig. 8) the potential rises to its full value in 8.8×10^{-5} sec., and to 91.3 per cent and 97.3 per cent of its full value respectively in 3×10^{-5} and 4.5×10^{-5} seconds. This residuum of delay of the rise is due, as has been explained above, to the capacity of the resistance box. As this is not used in the direct connection of the nerve to the input, there is even less distortion than the calibration curve indicates.

THE INPUT. An electrically heated incubator is used as the chamber for the nerve. The atmosphere is kept saturated by wet filter paper plastered against its walls. The temperature can be kept constant at 37° for mammalian nerve by means of the thermoregulator and low temperatures are maintained by filling the jacket with water at the desired temperature. The electrodes used are non-polarizable ones of the Zn-$ZnSO_4$-NaCl type. The nerve employed was usually the sciatic nerve of a large bull frog. The connections to the electrodes are at the cut end and the side, the nerve being killed between the electrodes by means of heat. The nerve is of such a length that there are still left five or six centimeters between the stimulating electrodes and the proximal lead. Connection is always made with the cut end to the grid. The lead to the grid is a lead-sheathed insulated copper wire. Both the sheath and the incubator wall with which it is connected are grounded. This was necessary because the input often picked up extraneous disturbances especially the one produced in the spreader circuit containing the abscissa plates. This disturbance, synchronized with the abscissa, amplified and connected to the ordinate plates, caused the line to deviate from horizontal in such a way that the deviation was greatest at first, returning logarithmically to the horizontal position. The presence or absence and the size of these extraneous disturbances depend upon the size of the leak from the first grid. The nerve connection as can be seen in figure 5

is in effect a grid leak and the disturbances are much less when the nerve is in place than when the only leak is the two megohms. The size of these disturbances undergoes variations not under our control, at times being very small. In an actual experiment the undeflected abscissa line is absolutely still and straight. The input connection to the ground is with ordinary annunciator wire. The connections are made through a double-pole-double-throw switch (S'', fig. 6), so that just after a record is traced a calibration curve can be made.

Calibration is effected by a constant current of short duration. A dry cell whose potential is 1.502 volts is connected in series with a 100,000-ohm resistance box and a rotating contact maker. The latter is the one used by Erlanger and Garrey ((6) and see I, fig. 6) in which a V-shaped conductor in a cylindrical wheel produces the desired current duration, the latter depending upon the rate of deflection of the spot. A fraction of the potential drop across the resistance box is led to the input through the double-throw switch (S', fig. 6) so that potentials of $+5$, $+10$ and $+15$ mv. may be impressed upon the grid. The contact maker is mounted on the shaft with the commutator wheel for producing the deviation along the abscissa and may be adjusted to the proper portion of the abscissa line. Due to its connections both to the commutator wheel and to the input it is apt to carry the logarithmic disturbance from the former to the latter. When this is the case the disturbance can usually be eliminated by using a lower resistance potentiometer, for instance one of 5,000 or 10,000 ohms instead of 100,000 ohms.

Another source of trouble has been the leads from the induction coil which, presumably acting as antennae, pick up extraneous disturbances. This was particularly true when long leads were used at the time that the elements of the apparatus were placed considerable distances apart. These disturbances can be eliminated by grounding one side of the secondary of the induction coil. The stimulus is the break induction shock from a Harvard (Porter) coil applied with platinum electrodes so placed that the stimulus starts at the pole nearest to the proximal lead. The break shock of this coil was found by Erlanger and Garrey (6) to be faster than that of any of the models studied by them.

Operation of the oscillograph. Since the life of a Wehnelt cathode is short in a tube containing gas the Braun tube is kept in operation only during the period of observation. Both the anode potential circuit and the filament circuit are connected through a double-pole-single-throw switch. To prevent arcing a 2,000-ohm resistance (R'', fig. 5) is inserted in series with the anode and the current in the

filament is built up slowly by means of an adjustable resistance whose intensity is measured by an ammeter. This adjustable resistance also is used to make the spot definite for definition is a function of the temperature of the filament. The filament current is increased until the spot becomes sharp. When any further increase is made the spot again spreads. The adjustment is very delicate and one hand is kept on the controlling rheostat during an observation.

In order to interpret properly the figures traced upon the screen of the tube an investigation of its coördinate system was necessary. In this investigation it soon became apparent that it would be necessary to differentiate between what we may call static coördinates and dynamic coördinates. As the latter are the only ones which are of use, they alone will be described.

It was first determined that in the range of the screen utilized the deflection of the spot both along the abscissa and the ordinate is a linear function of the potential. The central portion of the screen is the surface of a sphere whose center is in the region of the deflecting plates. The distances are measured on the surface of the tube and hence are segments of meridians of this sphere, equal in length for like potentials. When by means of impressing the spreader potential upon the horizontal plates ordinate potential lines are plotted at various points upon the X axis, they are found to be straight and at right angles to the latter in the working range but outside of this range they begin to diverge. In like manner when abscissae are plotted at the various ordinates, there is considerable convergence from left to right, increasing as the line deviates from the X axis. It is principally in this respect that the dynamic coördinate system differs from the static. The reason for the convergence has not been determined, it probably is dependent in some way upon the working connections of the tube. It can be seen in the calibration curve in figure 8.

As the deflection along any ordinate is linear, within the limits of the characteristic, the value of the potential at any point can be determined by means of the following expression:

$$e = \frac{y\,E}{Y - x\,tan\,\alpha} \tag{5}$$

where x and y are the coördinates, Y is the length of intercept of the calibration line upon the Y axis; α is the angle which the line makes with the X axis and E is its potential.

The value of the time at any point is determined by a rearrangement of (3), viz.,

$$t = C R \log_{\epsilon} \frac{X}{X - x} \tag{6}$$

Just after the tracing has been made of an action current a calibration by means of the constant current of short duration is made with the deflection in the same portion of the screen as the action current. After the tracing has been transferred by means of transparent paper, the

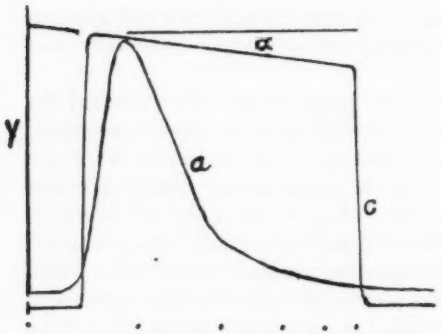

Fig. 8

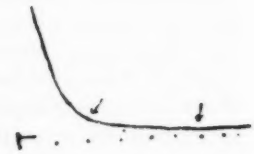

Fig. 9

Fig. 8. Braun tube record. Action current, *a*, from bull frog's sciatic. Temperature = 25.2°; X = 6.05 cm.; 1 mf.; 3000 ω; dots 1 σ apart. Calibration curve, *c*, with a constant current; *Y* = its intercept on the Y axis = 4.26 cm.; α = the angle the calibration curve makes with the X axis; tan α = 0.1428. Same nerve as figures 1 and 2. (Natural size.)

Fig. 9. End of the action current of figure 8; 1 mf.; 10,000 ω; dots 1 σ apart; first arrow at second notch, second arrow at end.

coördinates of the points on the curve are determined by mounting the transfer under a pointer carried on a mechanical stage of a type designed for microscopes. With the aid of the vernier the coördinates can then be read to 0.1 mm. The accompanying table (2) gives in detail the data necessary for plotting the action current in rectangular linear coördinates of potential-time. The data were obtained from the tracing reproduced in figure 8. As the end of the action current comes too near the end of the line in the record of figure 8, another record was made by setting the stimulus earlier and slowing the movement along the abscissa by inserting 10,000 ohms in the condenser circuit. Since the current has a

very gradual ending, an aid in locating the deviation from the base line is repeatedly to interrupt the stimulus, noting the point at which the base line changes its direction. It is from figure 9 that the data for the duration of the action current between the second notch (described below) and the end were obtained. The data are plotted in figure 10.

TABLE 2

X = 6.05 cm. R = 3050 ohms
Y = 4.26 cm. C = 1 mf.
E = 15 mv. $\tan \alpha$ = 0.1428

ABSCISSA	$\log_\epsilon \frac{X}{X-x}$	$1000\,RC \times \log_\epsilon \frac{X}{X-x}$	ORDINATE	$x \tan \alpha$	$Y-x \tan \alpha$	$\frac{y}{Y-x \tan \alpha} \times 15$
		(*time in σ*)				*millivolts*
0	0	0	0	0	4.26	0
0.47	0.081	0.247	0	0.07	4.19	0
0.62	0.108	0.329	0.03	0.09	4.17	0.11
0.75	0.131	0.399	0.15	0.11	4.15	0.54
0.85	0.151	0.460	0.49	0.12	4.14	1.77
1.05	0.190	0.580	1.82	0.15	4.11	6.58
1.15	0.210	0.640	2.73	0.16	4.00	10.24
1.20	0.221	0.674	3.12	0.17	4.09	11.44
1.28	0.237	0.723	3.58	0.18	4.08	12.86
1.38	0.258	0.787	3.73	0.20	4.06	13.78
1.51	0.286	0.872	3.64	0.21	4.05	13.48
1.60	0.307	0.936	3.46	0.23	4.03	12.88
1.85	0.365	1.114	2.89	0.26	4.00	10.84
2.15	0.438	1.336	2.12	0.31	3.95	8.06
2.45	0.519	1.584	1.45	0.35	3.91	5.56
2.65	0.576	1.757	1.02	0.38	3.88	3.93
2.81	0.624	1.904	0.77	0.40	3.86	2.99
3.05	0.701	2.139	0.60	0.44	3.82	2.36
3.50	0.863	2.631	0.37	0.50	3.76	1.47
3.83	1.002	3.057	0.22	0.55	3.71	0.89
4.16	1.163	3.548	0.17	0.59	3.67	0.69
4.91	1.668	5.087	0	0.70	3.56	0

THE ACTION CURRENT. The start of the action current is gradual; there is no evidence of a sudden or explosive change although the initial acceleration is very great. Heretofore this form of start has been obscured by the slowness and inertia of the recording mechanisms and by the small size of their deflections. Boruttau (7) in figure 3, plate II, pictures without comment such a start of the action current as recorded

by means of Hermann's rheotachygraph. A similar form of start appears in capillary electrometer records of the larger and slower muscle action currents.

To determine whether this form of start represents the process under the electrode only, an experiment was performed in which, in a nerve showing a large action current, a short heat block was produced just proximal to the proximal electrode. As a result of this procedure all trace of the action current disappeared; therefore there is no reason to believe that the current from the advancing wave of negativity contrib-

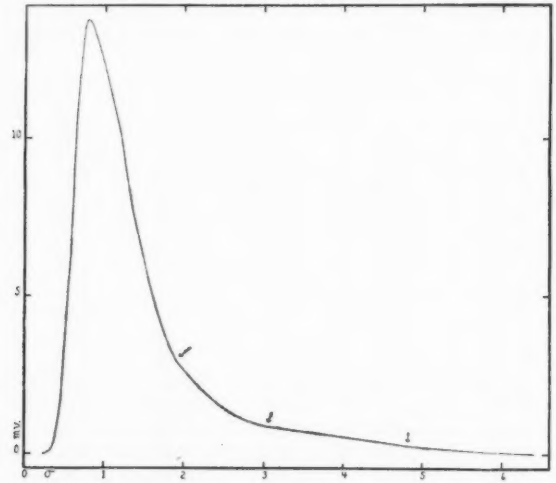

Fig. 10. The action current of figures 8 and 9 and table 2 plotted in rectangular linear coördinates. Abscissa: time in σ; ordinate: potential in millivolts. Arrows show the position of the three notches.

utes anything to the phenomenon until the process starts under the electrode.

The rising phase of the action current is smooth. The crest is reached in 0.54σ (fig. 8). In other nerves at about the same temperature the phase lasts from 0.54σ to 0.72σ (table 3). These are considerably longer durations than those obtained with the capillary electrometer. For instance, Gotch and Burch (8) found that the crest was reached in frog nerve in 0.5σ at 6° and Adrian (9) places the crest at 0.7σ at 13° in figure 5, and at 1.06σ and 0.47σ respectively at 6° and 16° in figure 6.

We thus find durations at temperatures close to 25° slightly longer than those of Adrian at 13° to 16° and of Gotch and Burch at 6°. At about 16° we have found values of about 1.5σ.

Without the accumulation of many data on frogs from various sources, and at different seasons of the year, it is difficult to make a comparison of one set of data with another. The longer anacrotic limb which we find must be due in part to the nature of the start of the wave. In previous methods this slow start being less apparent, the measurements were probably made from a point higher up on the rising wave. For example, in the nerve of figure 8 the rise from one-tenth of maximum to maximum is 0.36σ and in a nerve at 15° whose rising phase lasted 1.7σ the time from one-tenth of maximum to maximum is 1σ. The frogs used

TABLE 3

BULL FROG	TEMPERATURE	TIME TO MAXIMUM	MAXIMUM POTENTIAL	TIME TO FIRST NOTCH	TIME TO SECOND NOTCH	DURATION
	degrees	σ	*mv.*	σ	σ	σ
1	23.7	0.603	11.6	1.25	2.33	
2	11.0	2.03	6.1			
3	20.9	0.946	11.2			
4	15.0	1.71	15.36			
5	16.4	1.26	16.45	2.21	3.6	
6	26.4	0.598	14.6	1.27	1.889	7.86
7	25.0	0.721	14.3	2.38	3.87	
8	25.2	0.540	13.8	1.657	2.81	6.17
9	15.4	1.52	12.14	3.26	6.4	
Green frog						
1			23.6			
2			22.9			

in these experiments are accustomed to a warm climate. Their source is the region of New Orleans. The room temperature in St. Louis at the time of the experiments was 25° to 26°, the temperature of the water in the aquarium being about the same.

Garten (10) made string galvanometer records from four sciatic nerves of Rana esculenta. He made a rough correction of these curves according to the laws of the capillary electrometer. While this form of correction, as he realizes, is not justifiable, it gives results which are closer to the facts than the record itself, and which are in closer agreement with our results than the capillary electrometer data. He obtained at 18° a time to maximum of 0.9σ and at 32° of 0.55σ. We feel that our data on this point exceed in accuracy any previous data on account of

the large size of the deflections, the great rapidity of the movement along the abscissa and the freedom from distortion by inertia.

The maximum potential developed in the nerve of the bull frog (Rana catesbiana) has been found to be from 13.8 mv. to 16.45 mv. at temperatures of 16° to 25°. In the nerve of the green frog (Rana pipiens) the highest potential seen has been 23.6 mv. The potential recorded is, of course, not necessarily the potential developed at the seat of activity because the nerve can be considered as consisting of an active portion, or generator with high internal resistance, and of an inert portion which acts as a parallel conductor, thereby cutting down the potential drop across the leads.

The falling phase of the action current is much slower than the rising phase and its course is usually not smooth. The rising phase ends rather abruptly and it takes a definite short period for the decline in potential to attain its maximum velocity. The wave therefore is not symmetrical, but the peak is situated well over toward the side of the rising phase. There are quite regularly upon the curve of the decline of the wave points of more rapid change of direction, tending to produce notches, and in some nerves the intervals between these notches manifest themselves as definite waves with new maxima. There are quite regularly three such waves; in two instances we have seen evidences of a very small fourth wave. We have also seen these waves in the tibial nerves of the cat and rabbit, but never in the phrenic nerve of the dog. They cannot be attributed to a reduplicated stimulus by a shattered break because the records of the shocks show that they are single. Frog nerves vary considerably as to the degree in which they show this phenomenon. The waves appear in action currents from sub-maximal stimuli but are made more definite by increasing the strength of the stimulus. Figures 8 and 11 were taken in sequence from the sciatic nerves of different frogs but otherwise under as identical conditions as possible. In figure 8 the phenomenon is just developed; in figure 11 definite waves appear. A number of records were taken from the latter nerve using shocks of different strengths. In figure 12 the tracings obtained are drawn with their coördinate systems superimposed. With the weakest shock (curve with the dotted line) the notches are barely visible. The position of the first notch is indicated by the arrow. The stimulus as usual was the break shock of a Harvard induction coil operated by a single dry cell and, in this case, the secondary of the coil was set at the end of the slide and turned but a few degrees from vertical. Strengthening the shock by turning the secondary coil a few degrees

further from vertical accentuated the notches, and when the coil was turned to 60° from vertical the waves were well developed. The escape now lasted until the beginning of the action current and the first wave in this instance decreased in size. The total duration of the disturbance is not much increased by increasing the strength of the stimulus. It will be noted that the crests of the action currents come progressively earlier as the strength of the induction shock is increased. This probably means that the excitation process is completed sooner by the stronger and therefore steeper shocks.

The use of the Braun tube renders a study of the effect of the strength of shock upon the size of the action current very simple, because the escape does not alter the position of the crest of the action current. This has not been investigated in any detail but a number of observations

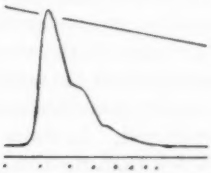

Fig. 11

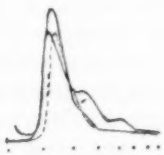

Fig. 12

Fig. 11. Braun tube record. Bull frog's sciatic showing well-developed catacrotic waves from a weak break shock. Calibration line at 15 mv. (Reduced one-half.)

Fig. 12. Three records of the same action current with different strengths of stimulation to show development of the catacrotic waves. Constants for figures 11 and 12: 1 mf.; 5000 $^{\omega}$; dots 1 σ apart; temperature 25°. (Reduced one-half.)

indicate that over a very short range of stimuli the size of the action current increases with the size of the shock. After this, no further increase in the size of the action current takes place, although the size of the escape becomes progressively larger. It encroaches higher and higher upon the rising phase of the action current, but the position of the crest does not change. These findings therefore confirm the conclusions which had been reached on this point from experiments made under more difficult conditions by Gotch (11) and particularly by Forbes and Gregg (12).

It is questionable whether the wavelets under consideration correspond to anything that has been previously described. It is now a well-known fact that muscle and nerve respond with rhythmical currents to stimulation by the make or break of strong constant currents. It has

further been found that the induction shock (6) may last so long that a nerve might respond with a second stimulation (Garten (12) and Forbes and Gregg (11)). We have one record made from the tibial nerve of a rabbit which was not responding normally, which shows a second wave and a shock of sufficient duration to produce it. This curve has the general form of Garten's figure 17 and Forbes and Gregg's figure 3B3, but not the form of the waves under discussion. There are other reasons for believing that these waves are not reduplications of the main wave.

a. In the first place they may be produced in some nerves in a well developed form by very weak shocks while in the phrenic nerve of the dog they did not appear after very strong shocks. *b.* Referring again to figure 2, which is the reproduction of the action current of a frog's sciatic, and the shock which produced it, the total duration of the shock as indicated by the escape is only 1σ and its effective duration must be much less. The current it produces reaches its crest in 0.54σ. In the light of Adrian's (9) observations on the relation of the refractory period to the action current it is highly improbable that the nerve could again discharge during the duration of the shock; nevertheless the waves in question are present though poorly developed. In other records, not reproduced, the duration of the shock is less than the rising phase of the action current. When the nerve is cooled the catacrotic notches are present although the ratio of the duration of the action current to the shock duration is higher.

c. When the intrinsic rhythm in nerve appears the individual waves have about the same period (Garten (13)). No evidence is found in our data that the waves have a common period. The time from the start to the first notch is always considerably longer than the time from the first notch to the second, even when the error in setting an exact location of the notches is discounted (table 3). The third wave is much longer than either the first or second but as it might be considered as the sum of two undifferentiated waves it is not justifiable to compare it with the former.

d. The double action current recorded by Garten was obtained with a make induction shock. Forbes and Gregg, likewise, state that deformation occurs at more remote coil distances with the make shock than with the break shock. Our waves are obtained with break shocks and the corresponding make to many of the breaks we are calling "stronger" is subminimal.

e. The extra wavelets appear more conspicuously in cold blooded nerve, although the refractory period is longer and the shock, due to

higher secondary resistance, is shorter. *f*. While the wavelets become more marked in preparations losing their irritability and in which the strength of shock is increased, some of our best instances were obtained in fresh and very irritable nerves with weak shocks.

While reduplication of the action current cannot be absolutely ruled out as the explanation, the evidence does not point in that direction. This being the case one must inquire into such possibilities as the summed effect of nerve components of different characteristics, or the production of the current itself by more than one process.

The total duration of the action current was determined in two nerves with considerable accuracy by placing the stimulus so early that the action current ended on the earlier and better portion of the line. The durations found were 7.86σ at 26.4° and 6.17σ at 25.2°. Many other slightly less accurate determinations agree with these. It is, however, difficult to compare them with the values obtained by other methods because of the various conditions of the experiments. The differential rheotome figures for the duration of the action current in frogs' nerve are as follows: Bernstein (14), 0.56σ to 0.8σ; Hermann (15), uncooled nerve, 5.03σ, cooled, 13.2σ; Head (16), winter frogs, 24σ, spring frogs, 7.90σ; Boruttau (7), 5σ to 7σ at 10° to 12°. With the capillary electrometer Gotch (11) obtained 9σ at 1.5° and Adrian (9), 9σ to 12σ at temperatures ranging from 6° to 16°. The many determinations obtained with the aid of the string galvanometer whether uncorrected or corrected by the capillary electrometer method while interesting cannot, for reasons mentioned above, be regarded as accurate. Considering the temperature at which our observations were made, it would seem that in general our durations are somewhat longer than others recorded in the literature. As the ending is very gradual and as its location can be determined definitely only with special precautions, it is likely that we have followed out the declining phase farther than these observers.

Since the impulse producing figure 8 traveled 5.6 cm. in 1.69σ, measuring from the beginning of the shock which is slightly previous to the excitation, the impulse was travelling at about 33.1 meters per second. The wave length, or the length of nerve in activity at any instant, is therefore 20.3 cm. and the crest of the wave is 1.77 cm. behind its start.

MAMMALIAN NERVE. A few records have been made from mammalian nerves. These were isolated, thoroughly freed of fascia, and mounted in the incubator which was maintained at a temperature of 37° and ventilated with oxygen. The whole process was carried out rapidly to keep the nerve as nearly normal as possible.

A rabbit tibial nerve was obtained immediately after death from concussion of the cervical cord. Figure 13 is a tracing of a submaximal action current. The end of the shock appears in the record. The wave has the same general form as the cold blooded action current except for the notches, but traces of these appeared with a very strong shock. The time to maximum is 0.24σ and the duration 2.05σ, although the end point is not precisely determined. The sharp bend on the declining phase comes at 1σ. A record of a maximal action current was made with a more rapidly moving spot (1 mf., 1000 ohms). The time to maximum was 0.25σ. These values of the time to maximum are con-

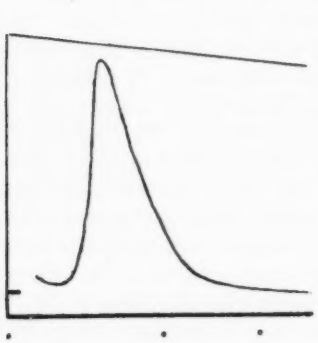

Fig. 13

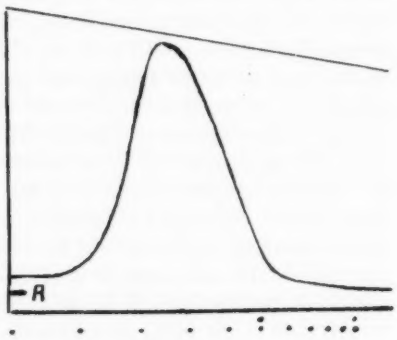

Fig. 14

Fig. 13. Braun tube record of rabbit's tibial nerve. 1 mf.; 2000 $^{\omega}$; dots 1 σ apart; temperature 36.6°; calibration line 15 mv. (Natural size.)

Fig. 14. Dog's phrenic nerve. 1 mf.; 1000 $^{\omega}$; dots 0.2 σ apart; double dots 1 σ apart. The distance between the line, *A*, and the start of the shock is the initial value of the logarithmic disturbance.

siderably shorter than Garten's approximations from string galvanometer records. He gives 0.5σ at 35° for the rabbit's sciatic and 0.7σ at 36° for the dog's sciatic.

The conduction time in this nerve as measured from the beginning of the shock was 81 meters per second. As excitation does not take place until somewhat after the beginning of the shock, the rate of propagation must be faster than this and approach the region of normal. The wave length is 16.6 cm. on the basis of the above figures and the crest of the wave is 1.94 cm. from the start.

A record from a phrenic nerve taken from a dog under paraldehyde anesthesia has the same general form as the tibial record except that

the notches did not appear with any strength of stimulus (fig. 14). In this record no trace of the producing shock is visible; the slant of the line is the logarithmic disturbance which we have described as being transmitted to the ordinate plates. The great speed of the spot makes this disturbance more evident than a similar disturbance would be in a slower record. Measurement can, however, be made from this line as a base line. The conduction time is 51.9 meters per second and the time to maximum is also slower than that of the tibial, being 0.36σ. Whether or not this is a physiological difference we are not prepared to say. The ratio of the two times to maximum practically equals the ratio of the conduction times.

SUMMARY

The low voltage cathode ray oscillograph offers a means by which nerve action currents can be easily and accurately recorded. A description is given of the apparatus by which this may be accomplished. It consists essentially of a three-stage amplifier, giving 7,000- to 8,000-fold amplification, working into a Braun tube. The method is possible because nerve action currents can be repeated with great precision twenty times per second and the record appears as a standing wave on the screen of the tube, where it may be drawn or photographed.

A description is given of apparatus for synchronizing the nerve potential changes recorded on the ordinate with the movement along the abscissa. The rate of movement along the abscissa is controlled by a condenser and resistance, and can be made very fast. The action current of mammalian nerve has been recorded in the range of velocities between 50 and 20 meters per second.

The method employed for connecting the nerve to the input is described; also the methods for calibration of the amplifier and Braun tube and for measuring the internal resistance of the nerve.

Records of frog and mammalian nerve are presented with data as to potentials and time relations.

The action current has a gradual start, a steep smooth anacrotic limb and a more gradual catacrotic limb. The latter like the former shows a period of great initial acceleration so that the crest is situated near the anacrotic side. In frog nerve and some mammalian nerves there are secondary waves on the catacrotic limb. Suggestions are made as to the cause of these waves.

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THE EFFECT OF SPLENECTOMY ON INTEGRATION OF MUSCULAR MOVEMENTS IN THE RAT

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Although there appear yearly a large number of papers dealing with the spleen, the physiology of that organ is *terra incognita* and our knowledge concerning the function of the spleen is at present not much further advanced than it was in the time of Malpighi. Indeed, the present conceptions concerning the physiological rôle of the spleen may be said to have no more weight than the fanciful ideas entertained by the ancients. Any experimental contribution on the subject therefore needs no apology for publication.

In connection with a study of the physiology of the prostate gland, one of authors in conjunction with J. L. Ulrich investigated the effect of prostatectomy on the integration of muscular movements in white rats (1). The results obtained in that investigation were very interesting and appear elsewhere. It occurred to the present authors that it might be worth while to employ the same method in connection with the extirpation of the spleen. In other words, that the effect of splenectomy on the integration of muscular movements might lead to some interesting results. Accordingly, the present experimental investigation was begun.

In the present series of experiments rats were trained to walk on the rope and their muscular coördination before and after splenectomy was carefully noted. The method employed was the same as that followed by Macht and Ulrich in connection with a study of prostatectomy and is briefly summarized as follows.

METHOD. A medium-sized cotton rope 1 cm. in diameter and 210 cm. in length is stretched tightly from one end of a room to the other at the height of about 6 feet from the floor. At each end of the rope is built a small wooden platform about 9 inches square, the surface of which is in the same plane with the rope. White rats are trained to cross the room on the rope from one platform to the other. The ex-

periment is begun by placing a dish of food on one platform and placing a rat on the rope a few feet away and allowing the animal to reach to the platform. On the following day the rat is placed on the rope at its middle, and on the fourth day the experiment can generally be begun by placing the animal on the platform at the other end and allowing the rat to cross over the entire length of the rope. This method allows a study of progression along a narrow path with the limbs of the animal converged inward and not separated in a natural way when progressing on a flat surface. All of the rats used in these experiments were from 45 to 50 days old at the beginning of the investigation. The animals were fed once in 24 hours after each trial on the rope. In the present investigation one trial a day was given to each rat, and it required on an average 50 days or 50 trials for each animal to master the problem completely and perfectly. At the beginning of training, progression along the rope is just barely possible with most of the rats. The animals make numerous slips, progression is arhythmic and the body is crouched and poorly adjusted to progression along the rope. There is usually marked trembling of the entire body musculature with a swaying of the hind quarters. Frequently loss in equilibrium occurs and the rat becomes suspended by the forelegs, from which position it readjusts itself with difficulty and crawls forward to the food platform.

On continuing the training rhythmic progression along the rope appears from the 15th to the 20th day of trial. This rhythmic progression is first noted in the front limbs while the hind limbs are lowered and are swaying. As the adjustment is bettered the hind quarters stand more firmly on the rope and are no longer crouching but are erect during the progression; so that after succeeding trials the animals run with both fore and hind legs erect, moving rhythmically, without slipping or loss of equilibrium. When this stage has been reached it is noticed that the body musculature is much firmer and better developed. All of the muscles, the abdominal and especially those of the limbs, feel harder and appear to have increased in tone. These changes in the muscles must take place for efficient adjustment of the body in order that progression may be rhythmic.

EXPERIMENTAL DATA. In the present investigation a total of 50 rats was included. Of these, 30 were splenectomized while 20 were used as controls. In the case of the controls some were left intact while others were operated on. The operation on the controls was laparotomy with an exploration of the viscera but without the extirpation of the spleen. In the case of the splenectomized animals 12 out of 30

were found to be unsuitable for study. Of these 12, 10 died from hemorrhage, pyogenic infections and other post-operative accidents while 2 recovered from the operation but owing to faulty operative technique showed adhesions and contractures of abdominal muscles and were therefore unsuitable for the study of muscular movements. The remaining 18 splenectomized rats recovered completely from the operation and were observed for a long time afterwards.

All of the rats were studied by the method outlined above. They were trained to cross the rope until they could do so without slipping or swaying. This training period generally consumed about 50 days or more. Having trained the animals to the best of their ability, the nature of the muscular integration and the running time across the rope were noted for a period varying from 10 to 20 or more days and the average running time was computed. The animals were then operated on and after recovery from the operation which occurred in from 1 to 2 weeks after the procedure, the rats were retrained for the rope for a brief period of time and then their muscular integration and running time were again noted. The results of the experiments are described below.

RESULTS. *Controls.* In the control animals, whether operated on or not, no change in the running time was noted. Indeed in some cases the running time of the animals became slower with their increase of age. A good illustration of a control experiment is given by the following protocol obtained in rat 40.

Control experiment

RUNNING TIME BEFORE LAPAROTOMY		RUNNING TIME AFTER LAPAROTOMY	
seconds		*seconds*	
7	7	7	6
6	5	6	6
6	Average 5.8	5	5
5		6	6
6		5	6
5		6	5
6		6	6
6		6	5
6		7	5
5		5	5
6		6	6
5		6	Average 5.8
6		5	

Other controls as, for instance, rat 41, gave an average running time before operation of 4 seconds, after operation of 4.5 seconds. Again rat 42 gave an average running time before operation of 5 seconds, after operation of 6.5 seconds. In none of the controls was there an improvement in the muscular integration or a shortening of the running time noted after the performance of laparotomy.

TABLE 1

Series I: Before splenectomy

RAT NUMBER	NUMBER OF SECONDS																MEAN AVERAGE
																	seconds
I	7	10	8	6	7	8	9	8	8	11	7	8	8	8	6	7	7.9
II	7	8	8	9	7	7	5	4	6	5	5	6	7	6	6	5	6.3
III	10	9	8	7	6	6	5	8	8	7	5	6	8	10	7	9	7.4
IV	14	8	9	15	10	11	7	8	7	8	8	9	10	8	8	9	9.3
V	10	12	10	15	10	20	10	8	9	10	11	9	8	10	10	11	10.8
VI	7	5	7	6	6	4	4	5	6	7	8	6	8	7	5	5	6.0
VII	5	8	7	4	7	8	5	5	4	6	5	4	4	5	5	6	5.5
VIII	4	5	5	4	5	3	4	5	5	6	5	7	5	6	4	5	4.9
Grand mean																	7.26

TABLE 2

Series I: After splenectomy

RAT NUMBER	NUMBER OF SECONDS																						MEAN AVERAGE
																							seconds
I	6	7	6	5	4	5	4	3	4	4	5	4	5	6	4	4	3	4	4	5	4	4	3	4	4.4
II	6	6	5	4	5	6	3	3	4	3	4	3	4	3	5	4	5	3	4	3	3	5	4	3	4.0
III	7	8	6	4	5	6	4	4	4	5	4	4	5	3	4	4	4	5	4	4	4	3	4	4	4.4
IV	6	6	4	5	6	4	4	5	6	5	5	5	5	4	6	5	4	5	6	5	5	5	6	5	5.1
V	9	8	6	5	5	5	6	5	4	6	4	6	6	4	6	5	5	4	6	6	4	6	5	4	5.4
VI	5	5	4	3	3	3	4	3	3	3	4	3	2		3	3	3	4	2	3	4	3	3	3	3.2
VII	5	4	3	3	3	4	3	2	3	Sick							Dead								3.3
VIII	5	5	3	3	3	2	3	3	2	3	2	3	3	3	2	3	3	3	3	3	3	4	2	3	3.0
Grand mean																									4.1

Splenectomized rats. The results obtained are tabulated as follows. The rats may be divided into two series, the figures obtained in series 1 are given in detail in tables 1 and 2. Table 1 gives the running time of rats 1 to 8 obtained on 16 successive days after the animals had been successfully trained to run over the rope. The mean average running

time for each rat is expressed in the last column. After taking the above 16 readings all of the rats were splenectomized and upon recovery from 10 to 14 days later the animals were retrained. The readings for 24 successive days are expressed in table 2. Here again the average running time is indicated in the last column. On taking the grand average for all 8 rats before and after splenectomy it will be noted that the running time before the operation was 7.26 seconds while the running time after splenectomy was 4.1 seconds. Careful observations were made on the character of the muscular movements and coördination of the animals before and after the operation. It was noted that after splenectomy many of the rats that were rather slow before were more

TABLE 3

Series II

RAT	BEFORE SPLENECTOMY	AFTER SPLENECTOMY
	seconds	*seconds*
IX	5.1	3.4
X	7.1	6.3
XI	7.2	5.3
XII	4.0	3.5
XIII	5.0	4.0
XIV	6.0	4.0
XV	9.0	7.5
XVI	6.5	5.0
XVII	10.0	7.0
XVIII	4.5	4.0
Average.....	6.45	5.0

lively and were holding their limbs more erect as well as exhibiting a faster speed.

In series B, 10 rats were studied in exactly the same way as in series A. Table 3 gives in brief the results obtained in the case of these animals. Here the average running time for 15 days before operation was 6.45 seconds and for 15 days after recovery from splenectomy was 5 seconds. It will be seen that in every case the running time was shorter after the operation.

COMMENT. The results obtained in the present investigation are rather interesting though their significance is not quite clear. When compared with the results obtained by the same method of experimentation in the case of prostatectomy these results are even more interesting.

Certainly the extirpation of the spleen is not as injurious to the integration of muscular movements of the rats as the extripation of the prostate. In making this statement only those animals are considered in which the operations of splenectomy and prostatectomy were perfectly successful. If we take the average running speed of all the 18 splenectomized rats studied in the present research, the average running time before splenectomy is 6.8 seconds while that after splenectomy is 4.6 seconds. These figures indicate quite distinctly an improvement in the speed of the animals produced by the operation and goes hand in hand with a better muscular integration observed in the muscles of the rats as they ran across the rope. The unexpected results obtained in the above experiments are of special interest in connection with an ancient belief concerning the relation of the spleen to the running power and endurance of man and animals, entertained by various ancient writers. It is well known to classical students that there was a belief prevalent that the extirpation of the spleen rendered runners more efficient. Thus for instance Pliny in his natural history states that the spleen—"Quamobrem inuritur cursorum laborantibus"—renders runners more efficient, and other references in ancient medical literature express the same idea. Thus the French have an expression "courir comme nun dératé"(3)—"to run as one with his spleen out"—and similar expressions are found in Germanic literature. The authors do not wish by any means to adduce the results of the above experiments as a complete vindication or corroboration of this ancient idea, but the parallelism between the results obtained in the experimental work and the results claimed by the older authorities is certainly of some historic interest.

CONCLUSIONS

1. The integration of the muscular movements and running speed were studied in rats by the so-called "rope method."

2. The effect of splenectomy on such rats was investigated.

3. It was found that the extirpation of the spleen did not interfere with the coördination of the muscles or the running time of the animals. On the contrary, the data obtained indicated that the muscular integration if anything was improved and the running speed was certainly shortened.

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STUDIES ON THE PHARMACOLOGY OF SODIUM CITRATE

II. The Influence of Sodium Citrate on Peristalsis

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Though citrates have long been employed as purgatives, experimental studies on their mode of action are of comparatively recent origin. Indeed it is only within the last two decades that observations on this subject have appeared. Early in the century MacCallum (1) was the first to report experiments designed to throw some light on the mechanism by which sodium citrate stimulated intestinal activity. The results he obtained, using strong solutions, indicated that the salt caused powerful contractions of the isolated intestines of the rabbit. Later, Auer (2) studied the effect of subcutaneous and intravenous injections of citrate into rabbits and also produced increased contractions of the intestine, especially of the duodenum. He stated, however, that the movements of other parts of it were not affected and that he was unable to cause purgation. These experiments were repeated by Bancroft (3) but his findings were at variance with those of Auer. The latter (4) reinvestigated the subject and corroborated his previous results. Observations on the action of citrate on the intestine of the rabbit were also made by Salant and Wise (5). They found that large doses given intravenously had no effect, but subcutaneous injections were sometimes followed by diarrhea. Salant and Schwartze (6) studied its action on segments of various parts of the intestine of rabbits and cats suspended in Locke's solution containing different amounts of the salt. Their experiments on the rabbit's intestine showed that low concentrations stimulated the small, but not the large intestine, while strong solutions caused depression in both. In experiments on the intestine of the cat, they observed that every part may be depressed even by low concentrations. From this brief review of the results of different investigators, it is evident that they were not in agreement as

to the action of citrates on the intestines. In this connection attention may be called to the methods which they employed in these studies. With the exception of the last-mentioned investigation, all the observations which they reported were made by direct inspection. While some information thus obtained may prove to be of considerable interest, it is evident that the results will be of greater value if more accurate methods are employed. The subject was, therefore, investigated by us de novo by means of the graphic method but the observations were greatly extended. They include studies on the effect of sodium citrate on different parts of the intestine in situ, an inquiry into the mechanism of its action, tests on the uterus to ascertain the reaction of smooth muscle structures other than the intestine and experiments on segments of the isolated intestine suspended in Locke's solution.

Methods. After numerous trials, we found that the Trendelenburg (7) method was best suited for recording the contractions of the intestine in situ. It proved so satisfactory in these experiments that we used it also for recording uterine contractions. Cats, dogs and rabbits were the subjects of the experiments with urethane as the chief anesthetic. Chloretone was used in a few dogs, while some experiments were carried out on decerebrated animals. The methods used in observations on the isolated intestine were essentially the same as those employed by Salant and Schwartze (6). Three per cent sodium citrate, which is approximately isotonic with the blood, was injected intravenously or 20 per cent intramuscularly. The chemically pure salt was used and was obtained by recrystallization of the better grade found on the market.

Results. It may be stated at the outset that the intravenous administration of sodium citrate was usually followed by marked and, with some exceptions, prompt augmentation of the motor functions of the intestines, either greatly increased tonus or stimulation of the rhythmic contractions or both being observed. But the response of the intestine in different animals indicated that the reaction was not exactly the same in all. As will be shown below, the results obtained showed appreciable differences in each of these animals. When citrate was given to cats, tonus and the rhythmic contractions were greatly increased. Even small amounts, 10 to 15 mgm. of the salt per kilo, provoked vigorous movements, but the effect was much greater when large doses were injected, 30 to 60 mgm. citrate per kilo, causing much stronger contractions which lasted 3 to 9 minutes. The stimulating effect was even greater in some experiments when the injection of the larger doses was repeated, but in most cases no marked differences

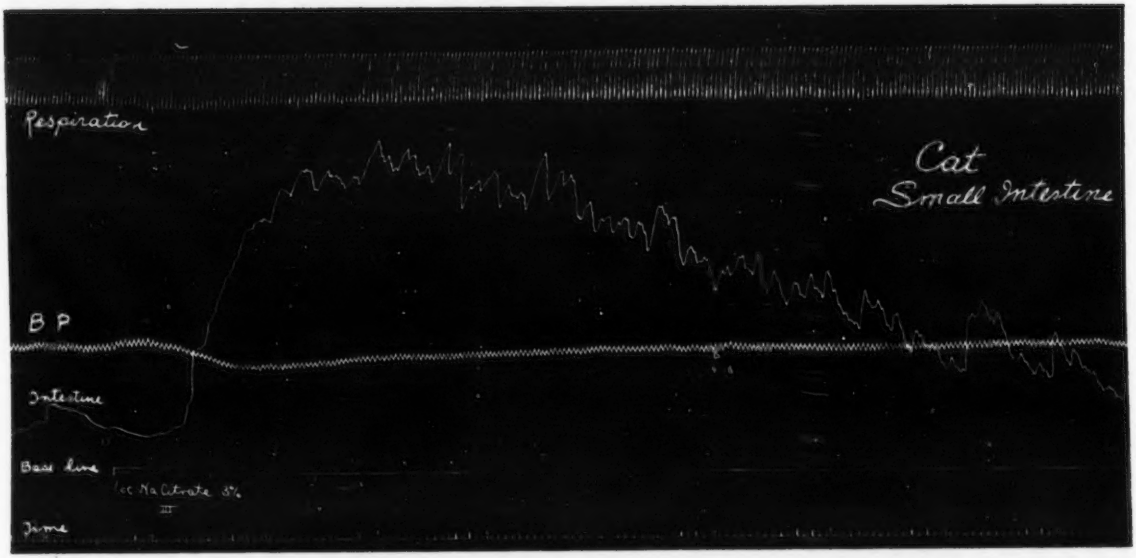

Fig. 1. The action of sodium citrate on small intestine of the cat: The injection of 30 mgm. sodium citrate intravenously into a cat weighing 2.1 kilos produced a marked increase in tonus and rhythmic contractions, the effect lasting about 9 minutes. Little effect on circulation and respiration. Time—4 seconds and 1 minute. Reduced one-half.

could be observed though the intervals between the injections were only 10 or 20 minutes. It is of interest to note in this connection that depression of the intestine was never observed even when the injections were much more frequent; this, it may be recalled, being in striking contrast to the action of citrate produced upon the circulation and respiration by Salant and Kleitman (8). Sodium citrate likewise stimulated the intestine in dogs, but in these animals tonic contraction predominated, the rhythmic movements being only moderately increased or not at all. The effect was sometimes marked when the injection of the same dose was repeated but the augmented action was much greater when briefer intervals were allowed between injections.

The results obtained in rabbits were different from those observed in carnivora. Well-marked stimulation of the small intestine was produced in one experiment by each of ten injections of 30 mgm. citrate per kilo given at intervals of 6 to 21 minutes, but the action was of shorter duration than that which was observed in cats and dogs. The same dose likewise caused stimulation in two other rabbits. The effect was reversed, however, after a few injections. Complete relaxation with abolition of all movements of the intestine for 1½ minutes was observed in one rabbit and transitory relaxation immediately after the injection occurred in another. The other effects, stimulation of respiration and a fall of blood pressure, were the same as were observed in previous experiments with sodium citrate. Typical experiments illustrative of the effect produced in different animals are submitted.

Experiment 138. Cat weight 2.25 kilos, urethane anesthesia. Three per cent sodium citrate was injected intravenously at intervals of 6 minutes to 1 hour; dose, 60 mgm. sodium citrate per kilo. The first injection caused strong contractions in the previously quiescent intestine, the effect lasting several minutes. The next dose, given 15 minutes later, produced the same effect but this was greater after subsequent injections, which were made at shorter intervals. A notable difference, however, was observed in the response caused by earlier injections and those given after a considerable amount of citrate was introduced. While stimulation of tonus marked the action of the former, increase of the rhythmic contractions was the main effect produced by the latter.

Experiment 140. Dog, weight 2.31 kilos; urethane anesthesia. Three per cent sodium citrate was injected intravenously. The first dose of 10 mgm. citrate per kilo caused a slight stimulation of tonus of the small intestine for a brief period. The same amount given 5 minutes later

likewise stimulated the tonus and also increased the frequency of rhythmic movements. The dose was then increased. Thirty milligrams citrate per kilo injected a number of times at intervals of 6 to 53 minutes caused tonic contractions, each lasting a few seconds. Stimulation was somewhat greater when the dose was doubled and

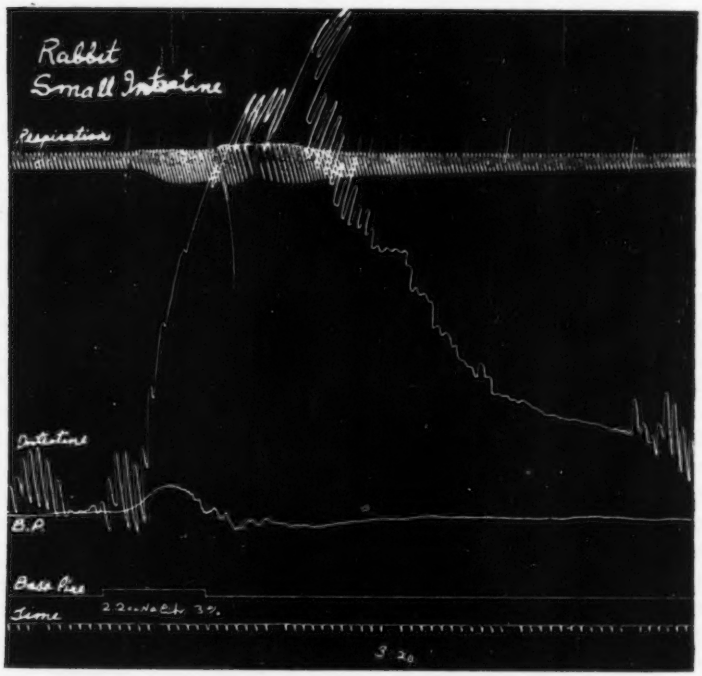

Fig. 2. The action of sodium citrate on small intestine of rabbit. The intravenous injection of 66 mgm. sodium citrate into a rabbit weighing 2.2 kilos produced an enormous increase in tonus and rhythmic movements lasting about 5 minutes. Time—5 seconds and 1 minute. Reduced one-half.

injected 9 to 24 minutes apart but the contractions were more pronounced and were longer in duration (8 minutes) when the same amount of citrate was injected after an interval of 83 minutes. The results obtained in these experiments showed that the effect of citrate decreased with the frequency of administration.

Experiment 182. Rabbit, weight 2.1 kilos; urethane anesthesia. Three per cent sodium citrate was injected into the jugular vein. Ten doses of 30 mgm. citrate per kilo each were given at intervals of 5 to 22 minutes. Increased contractions preceded by temporary relaxation of the intestine were observed. Tonus and rhythmic movements were stimulated by the first dose and more by the second for about 3 minutes, but the effect was less after subsequent injections. Practically the same effect was produced when the amount of citrate was increased to 60, then to 90 mgm. per kilo. Blood pressure was lowered and respiration was stimulated after the injection of citrate.

Observations were also made on the action of citrate on the cecum and the large intestine. Stimulation for 1 or 2 minutes preceded by transitory depression occurred after the injection of 30 mgm. sodium citrate per kilo in one experiment on the cecum. The same amount per kilo of body weight failed to produce any effect on the colon but doses of 60 and 90 mgm. citrate per kilo caused very strong tonic contractions without producing at first any changes in the rhythmic movements. We noticed, on the contrary, that the amplitude as well as the rate was temporarily diminished but as the tonus effect subsided the rhythmic movements became very forcible. The duration of the effect was somewhat longer than in the small intestine and in one experiment the increased movements lasted as long as 30 minutes. Another noteworthy difference between the two may be recorded. It will be recalled that after the injection of sodium citrate, the latent period of the small intestine was invariably short, seldom exceeding a few seconds, whereas the response of the large intestine occurred after a lapse of 1 or 2 minutes. Important also is the fact that the small intestine of the cat responded to smaller doses while the reaction of the large intestine to the same amounts was not constant. Studies on the action of sodium citrate on the intestinal movements were also made when a solution of the salt was given intramuscularly. Ten cubic centimeters of a 20 per cent solution were injected into the gluteal muscles of a dog weighing 8 kilos. Increased tonus and augmentation of the rhythmic movements set in within 2 minutes and lasted fully half an hour, the increase in peristaltic activity being most marked during the first 10 minutes after the injection. Another experiment was performed on a cat weighing 2.75 kilos, the same amount of the salt was injected into the gluteal muscles. No effect on the intestine could be observed at first but within 5 minutes stimulation set in and gradually increased, the maximum effect being attained in 22 minutes after the injection and lasting 35

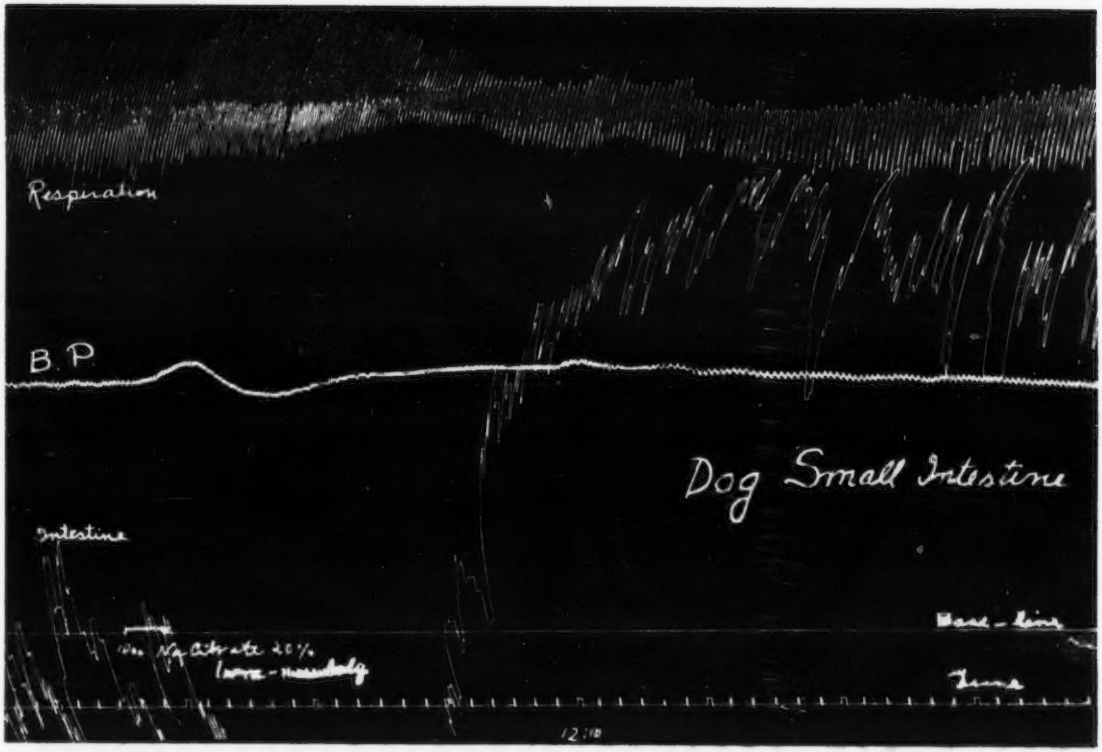

Fig. 3. The effect of an intramuscular injection of sodium citrate. Dog weighing 8 kilos received 2 grams sodium citrate into the gluteal muscles. Two minutes later the tonus of the small intestine was markedly increased. Respiration stimulated immediately, but little effect on blood pressure, showing that no citrate entered a vein. Time—10 seconds and 1 minute. Reduced one-half.

minutes when the increased movements began to subside. Increased contractions could still be noticed, however, 2 hours after the injection. A second dose of 2 grams of citrate was given after an interval of 3 hours. The increase of tonus was less pronounced and the contractions were less frequent. The effect lasted about 1 hour and the cat died. It is of interest to note that 48 minutes after the first injection the cat passed soft feces, thus indicating a tendency to purgation after the intramuscular injection of sodium citrate. The results presented above thus corroborated those of previous investigators, whose observations were made, however, by direct inspection. But we have also shown that the effect of citrate on the intestine was of short duration and that stimulation was produced by different amounts injected at various intervals. In this connection attention may be called to the effect of sodium citrate on the isolated intestine. Salant and Schwartze (6) observed that medium concentrations stimulated the small intestine of the rabbit but usually depressed that of the cat, while the large intestine was depressed in both by all concentrations. We repeated the experiments on the small intestine and corroborated their observations. The contradictory results obtained in experiments with citrate on the intestine in intact animals and in vitro will be discussed later.

We also tested the action of sodium citrate on smooth muscle structures other than the intestine. Several experiments carried out with different amounts injected intravenously failed to show any noticeable changes in the contractions of the uterus in situ. Having established that citrate stimulated the contractions of the intestine in different animals, we carried out the following experiments to determine whether the increased movements were caused by direct action on the muscle substance or indirectly by stimulation of the endings of the motor nerve fibers. Sodium citrate injected intravenously into a dog produced tonic contractions but the rhythmic movements of the intestines were hardly affected. This was followed by a dose of atropine just sufficient to paralyze the cardiac vagus. After a brief interval the same amount of sodium citrate was injected as before. Tonus and the rhythmic movements were stimulated but the effect was less than after the first dose. The results obtained in several other experiments were more decisive as the injection of citrates after atropine failed to produce any response of the intestine, thus showing that the contractions were due to stimulation of the peripheral nervous mechanism and not to action of the muscle substance. This was further shown when citrate was injected after the effect of atropine was antagonized by pilocarpine.

An illustration of the mechanism of the action on the intestine is shown by the following experiment.

Experiment 175. Cat, weight 2.3 kilos; urethane anesthesia. Two injections of 30 mgm. sodium citrate per kilo, each, were made 10 minutes apart. Reaction was prompt and very strong. Both vagi were then divided and the peripheral end of the right vagus was stimulated by the faradic current with the usual effect on the heart. A dose of 0.4 mgm. atropine sulphate was injected 3 minutes later and the peripheral end of the right vagus was stimulated again but no reaction was obtained. Thirty milligrams citrate per kilo injected three minutes after the atropine caused a fall of blood pressure and respiration was stimulated but the intestine did not respond. When the same dose was given again 5 minutes later, the results were likewise negative. Several injections of pilocarpine failed to induce intestinal movements but after the repeated administration of large doses, vigorous contractions reappeared. The injections of citrate were then resumed, the same doses being given as before. The first injection had no effect on the intestine but later injections stimulated the contractions as before the administration of atropine. It is obvious from this and other experiments that the motor effects of citrate on the intestine depend upon the condition of the nerve endings, for when these are depressed or paralyzed by atropine, citrate is no longer effective. Stimulation may be produced, however, when the peripheral endings regain their irritability under the influence of pilocarpine. It may be stated in this connection, that the irritability of the muscle was not changed by atropine for the intestine contracted after the injection of barium chloride. Although citrate stimulated the intestine after atropine when followed by pilocarpine, we noticed that this was not always the case. Relaxation instead of contraction sometimes occurred instead. This reversal of the action of citrate will be fully discussed in a forthcoming communication.

Although the data presented above point to the action of citrate on the peripheral nervous mechanism of the intestine, the evidence may not be regarded as entirely conclusive, for we found that atropine sometimes failed to depress or paralyze the endings of the motor nerve fibers in carnivora. Indeed, we observed that it, on the contrary, stimulated the intestinal contractions. This happened, however, very seldom in intact animals and though it occurred more frequently in the isolated intestine, it may be safely stated that stimulation of the contractions of the intestines by atropine was very exceptional. We ob-

served it in a few experiments only on cats and dogs and never in the intestine of the rabbit. Hirz (9) also reported that atropine stimulated the intestine in some cats but caused depression or paralysis in the intestine of the rabbit. The evidence presented, therefore, warrants the conclusion that sodium citrate primarily stimulated the endings of the motor nerves of the intestine, for it failed to stimulate the intestine in intact animals or in vitro when these were previously acted upon by atropine.

The reaction of the large intestine to citrate is of interest in this connection. It will be recalled that its latent period was considerably longer and that its contractions, after small doses, were not constant and were weaker than that of the small intestine. This may be explained by the observations of Bayliss and Starling (10) who found that the activity of the local nervous mechanism was diminished from the ileo-cecal valve downward and that inhibition was more readily produced than excitation. Hence it would seem that the reaction of the small and of the large intestine to citrate corresponded to a difference in the irritability of the local nervous mechanism in the two showing that the citrate acted upon the nervous structures and not upon the muscle fibers. This would also explain the negative results which we obtained in experiments with sodium citrate in the uterus. If the effect of citrate on the intestines were due to stimulation of the muscle substance, increased contractions of the uterine muscles would also occur for there is no reason to assume that the smooth muscle differs so radically in structure and composition in both organs that the substance which causes a powerful stimulation in one would be ineffective in the other. Mention was made above of the effect produced by citrate on the isolated intestine and when it was introduced into the circulation. The contradictory results—relaxation in one and contraction in the other—may be explained on the assumption that the different effects were caused by increased irritabilty of the inhibitory mechanism after removal of the intestine from the body. That depression as well as stimulation depending upon the physiological condition of an organ may be produced in response to the same stimulus is well illustrated by the action of adrenalin on the pregnant and non-pregnant uterus. Perhaps a similar mechanism is at work in the intestine.

SUMMARY

1. Intravenous and intramuscular injections of sodium citrate stimulated the motor function of the intestines but its action on the small

intestine was greater than on the large intestine, the amount required to stimulate the contractions being less, the latent period being shorter in the former than in the latter.

2. The action of citrate also varied in different animals. Stimulation of tonus predominated in dogs' intestine; augmentation of the rhythmic contractions was largely observed in the rabbit while both occurred in cats.

3. Experiments with citrate on the isolated intestine indicated that stimulation was the main effect in the rabbit's intestine and depression the most frequent result in the cat's intestine.

4. Citrate had no effect on the uterus in situ.

5. Evidence was produced showing that stimulation of the intestine in intact animals by sodium citrate was due to its action on the endings of the motor nerve fibers and not on the muscle substance.

6. It was suggested that the depression of the isolated intestine might be due to stimulation of the inhibitory mechanism.

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THE UTILIZATION OF OXYGEN IN THE BLOOD AT DIFFERENT STAGES OF ANOXEMIA

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The arterial blood of a normal man breathing atmospheric air is approximately 95 per cent saturated with oxygen. The venous blood under the same conditions has an oxygen content of four or more volumes per cent less than the arterial blood. The general observations presuppose a number of different factors controlling the physiologic mechanisms. These we know vary greatly in the same individual. The causes of the variations in the oxygen content of the venous blood have been discussed in detail by Lundsgaard (15). The outstanding factors that should be considered are, *a*, the composition of the air breathed; *b*, the hemoglobin content of the blood; *c*, the circulatory efficiency; and *d*, the pulmonary efficiency. The circulatory constants of rate and volume and the diffusion rate of oxygen in the peripheral tissues control in no small degree the difference between the volumes per cent of oxygen in the arterial and venous bloods. The oxygen content of the arterial blood is itself conditioned by the hemoglobin content of the blood, the composition of the air breathed, the volume of air passing over the epithelial membrane each minute, and the oxygen diffusion constant of the alveolar membrane.

In progressive anoxemia, induced experimentally, the oxygen content of the air breathed is gradually lowered until the amount absorbed fails to support the physiologic activity of the tissues. An animal responds to anoxemia by extensive and complex compensatory changes. Certain of these responses have been described in a series of papers in the last five years. The references to the literature have been recently presented (6), (7), (8), (9).

In a previous paper (10) we reported the changes in the oxygen content of the arterial blood of dogs during progressive anoxemia.

We here report the corresponding changes in venous blood, based on analyses made coincidently with those of the arterial blood. Our object has been to determine the changes in the utilization of oxygen from the blood, and if possible to associate these changes with the coincident compensatory changes in the circulatory and respiratory mechanisms.

Increased respiratory volume compensates for oxygen want only to the extent to which the alveolar oxygen tension may be made to approximate the oxygen tension of the air inhaled. As the alveolar oxygen tension progressively falls the oxygen tension of the arterial blood is correspondingly reduced. An augmented minute volume flow of blood is the only compensation possible on the vascular side of the absorbing membrane. The passing of the maximal amount of venous blood through the maximally ventilated lungs thus maintains the greatest possible diffusion pressure of oxygen between the alveoli and the blood. It insures the maximal absorption of oxygen for each unit of time. Notwithstanding these two types of mechanical compensation in the respiratory and vascular systems the oxygen tension falls in the arterial blood, though not quite so rapidly as it would without the compensations.

The oxygen tension of the venous blood is also progressively reduced. The effect of the venous oxygen reduction is to lower the oxygen reserve, a reserve expressed in the normal individual by a venous oxygen content far above the level of oxygen exhaustion. Apparently there is a wide margin of safety in the venous oxygen content. This has already been indicated (6) by the fact that the basal rate of oxygen consumption continues surprisingly uniform during the development of anoxemia. It is obvious that the venous oxygen cannot be maintained beyond the stage of anoxemia at which the oxygen utilization coincides with the arterial oxygen content.

Krogh (11), (12) has discussed in detail the oxygen supply to the tissues. In the resting muscle of the guinea pig the oxygen tension is normally 0 mm. During muscular work the oxygen tension of the muscular tissue was approximately that of the venous blood. The required pressure difference between the blood and tissue, to secure an adequate supply of oxygen to the latter, was very low, the calculated maximum being but 6 mm. for the guinea pig and somewhat less for the dog. Unless the oxygen tension of the arterial blood falls below this critical level, the diffusion pressure in the blood will be sufficient to provide for the oxygenation of the tissues. In order to maintain the basal rate of oxygen consumption, any further decrease in the oxy-

gen content of the blood below the critical pressure level necessitates proportional increase in the volume of flow. Such compensation is restricted to very narrow limits. The absolute compensatory limit is reached when the blood flow under the maximal compensation is inadequate to supply the normal volume of oxygen for tissue metabolism. Beyond this limit the oxidation rate in the tissue will fail rapidly and permanent anoxic tissue asphyxiation result.

Greene and Gilbert (7), (8), (9) have described the respiratory responses and the profound cardiac and vascular functional changes in what they call the post-crisis stage. The maximal vascular changes take place somewhat later than the respiratory. When respiration stops it is obvious that the arterial blood will be quickly deprived of its oxygen; in fact little more than a trace of oxygen remains in the blood after the crisis.

Methods. The experimental procedures and the analytic methods used in the two series of experiments were discussed in detail in the paper published in 1922. The venous blood samples were drawn from the jugular vein at the same time that the arterial blood and the alveolar air samples were taken. The blood was drawn under oil and care was taken to prevent stasis at the time of drawing. The venous samples were analyzed immediately in duplicate by the Van Slyke blood-gas apparatus and method. In the second and best series the alveolar oxygen sample was taken from a sound inserted deep into a bronchus, as previously described.

Experimental data: the venous oxygen content. It is recognized that the oxygen content of the venous blood depends, in great degree, on the rate of blood flow. This may be subject to wide variation. Meakins and Davies (16), Adams and Morris (1) and Barcroft and Nagahashi (3) have recently emphasized the great changes in the venous blood of the extremities as a result of changes in temperature and consequent variations in the rate of blood flow. Means and Newburgh (17) and Lundsgaard (14) have emphasized the close parallelism, in normal individuals, of the oxygen content of the venous bloods from the arm and the right ventricle. The jugular blood has a greater degree of saturation than that in the ventricle, nevertheless if the experimental conditions are kept uniform, the changes in the oxygen content of the jugular blood may be taken as an index of the changes in the oxygen content of the mixed venous blood.

The oxygen content of the jugular blood of dogs breathing atmospheric air varied from 7.5 to 18.4 volumes per cent. This corresponds

to a saturation of from 53 to 76 per cent. These values are in approximate agreement with the values found by Lundsgaard (14) in the venous blood from the cubital veins of normal men.

The entire data from the various experiments are shown in table 1. With the progressive induction of anoxemia the venous oxygen falls rapidly. The exact measure of this change is best shown graphically in figure 1. The venous blood shows considerable variation. The explanation of some of the variations will be discussed later. In general, however, the venous saturation parallels the arterial saturation and the standard dissociation curve.

Oxygen utilization. Accurate definition of the terms used in the discussion of the changes in the oxygen content of the blood during its passage through the tissues is desirable. The difference between the oxygen content of the arterial and venous bloods is a measure of the oxygen absorbed by the tissues during the passage of the blood through the capillary system, that is, the *oxygen utilization.* The portion of the hemoglobin actually utilized for the transportation of oxygen to the tissues is indicated by the relation between this oxygen utilization and the oxygen capacity of the blood, that is, *the percentage utilization of the hemoglobin.*

Various synonyms have been used to indicate the difference between the oxygen content of the arterial and venous bloods; oxygen loss, oxygen absorbed, oxygen difference, oxygen used, and so forth, but we have preferred the term oxygen utilization, emphasizing the relation of the changes in the blood to tissue respiration.

The term "coefficient of utilization" used by Krogh and Lindhard (13) does not express the relation of the oxygen transportation to the oxygen capacity as well as does "percentage utilization" and the latter is accordingly used. Lundsgaard (14) first introduced the term "oxygen unsaturation," with particular reference to the oxygen content of the venous blood. As is emphasized in his definition of the term, it is not to be used as a measure of the oxygen utilization because of the possibility of variation in the degree of saturation of the arterial blood.

The oxygen utilization in the blood depends on two factors, total oxygen consumption, and rate of blood flow. The rate of blood flow through the lungs is subject to wide variations, depending on the supply of venous blood to the right heart and the heart rate. The maximal variation occurs under conditions of muscular work, but if consecutive determinations are made on a normal individual under constant conditions, the blood flow is remarkably constant. In our experiments, external influences were carefully eliminated.

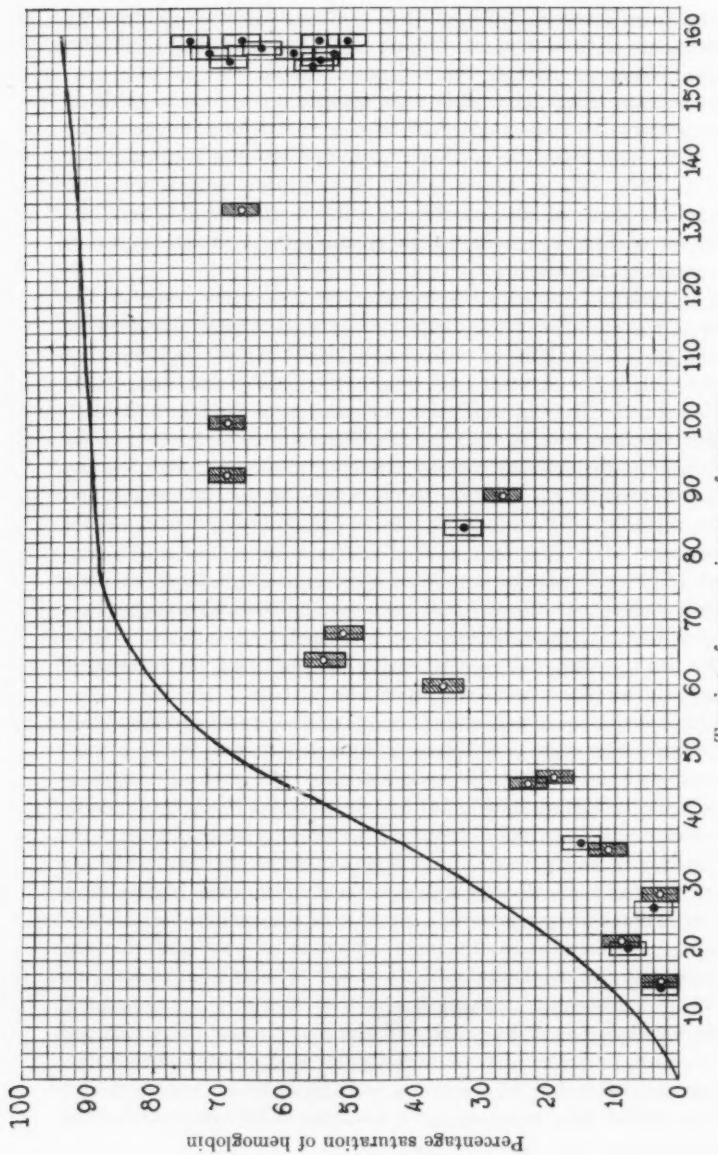

Fig. 1. Ordinates, percentage of saturation of hemoglobin in venous blood. Abscissae, tension of oxygen in millimeters of mercury. Dissociation curve of dog's blood at 40 mm. tension of carbon dioxide. The cross-hatched rectangles denote determinations plotted at oxygen tension of alveolar air. The plain rectangles denote determinations plotted at oxygen tension of inspired air. The area drawn around the point is the experimental error in each case.

The changes in the oxygen utilization in a typical experiment on dog 9 are given in table 2. These results are shown graphically in figure 2 in order to illustrate the relationship between the arterial and venous blood. The total height of the column indicates the arterial saturation at the different alveolar oxygen tensions. The shaded area represents the venous saturation while the unshaded area represents the percentage utilization of the hemoglobin. As pointed out in the preceding paper, the arterial saturation decreases with progressive anoxemia, the fall paralleling the oxygen dissociation curve. The venous oxygen, which represents the reserve oxygen supply, decreases with the reduction of arterial saturation. In the last two experiments plotted, the venous blood contains a smaller amount of oxygen than can be accurately estimated in the apparatus used, hence may be considered as practically, if not absolutely, anoxic. The oxygen utilization is nearly constant throughout the greater part of the curve. With the development of complete venous anoxemia, the percentage utilization approximates the arterial saturation.

The curve shows a slight increase in the percentage utilization when the alveolar oxygen tension is reduced to about 40 mm. Respiration studies reported previously (6) do not show changes in the metabolic rate during the progressive induction of anoxemia. A long series of experiments on the same animal is followed in time by a lowering of the blood pressure. Study of table 2 indicates that the changes observed in the blood oxygen utilization in dog 9 must be referred, in part at least, to changes in the rate of blood flow incident to the lowering of the blood pressure. The final entry on the chart is taken from experiment 47 on dog 11 in order to illustrate the condition of oxygen in the blood during the post-crisis stage of the reaction.

In table 1 the last two columns show the data on the oxygen utilization for the entire series of experiments. These data are presented graphically in figure 3. The oxygen utilization in animals breathing atmospheric air varied widely from animal to animal with an average of 6.45 volumes per cent. This corresponds to a mean percentage utilization of 30. The initial degree of oxygen utilization was maintained with little or no change during the greater part of the experimental series. This is best shown in the series of experiments on dog 9, referred to in table 2. When the alveolar oxygen tension was reduced to between 25 and 35 mm., the venous blood was nearly completely deoxygenated, the oxygen utilization approaching in value the total arterial content. With further reduction of the alveolar oxygen the

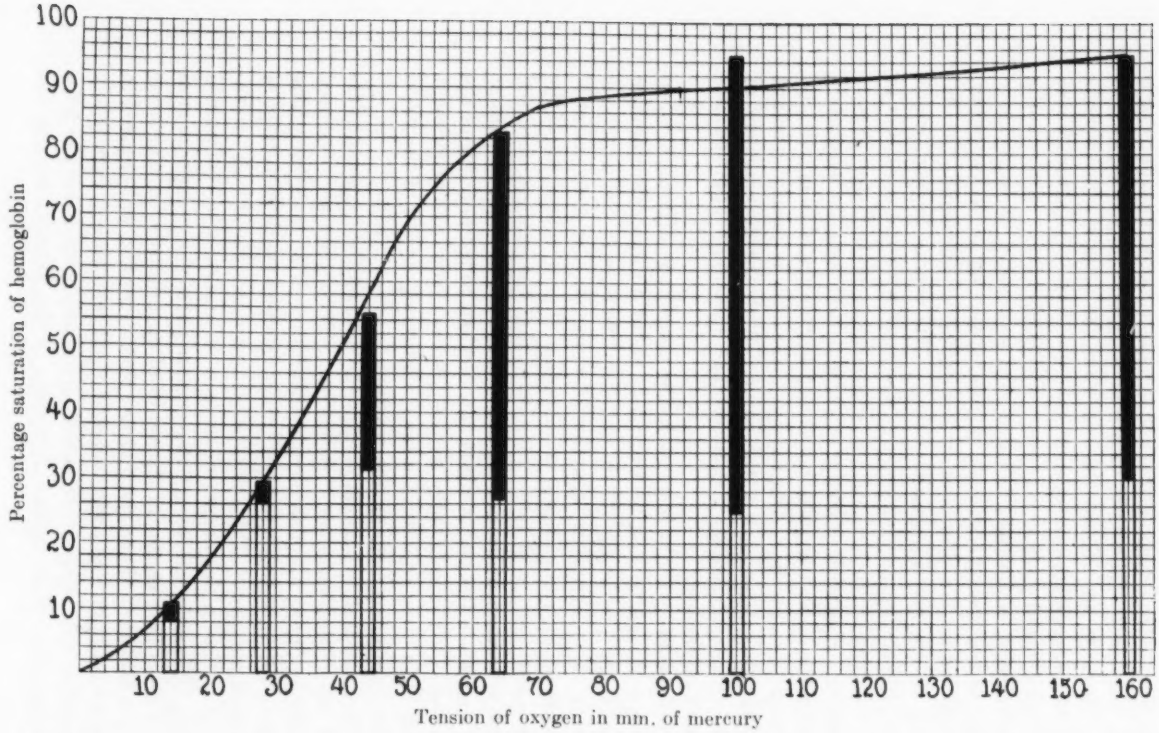

Fig. 2. Ordinates, percentage of saturation of hemoglobin. Abscissae, tension of oxygen in millimeters of mercury. Dissociation curve of dog's blood at 40 mm. tension of carbon dioxide. The total length of the column denotes the percentage of saturation of the arterial blood. The shaded rectangle denotes the percentage of saturation of the venous blood. The plain rectangle denotes the percentage utilization of the hemoglobin. The determinations are plotted at the oxygen tension of alveolar air. The relative constancy of the percentage utilization of the hemoglobin at different oxygen tensions is emphasized.

percentage utilization is rapidly reduced to zero. The arterial oxygen is quickly exhausted at this stage, at least to an amount within the limits of accuracy of the analytical methods. Oxygen is no longer available in the arterial blood in the amount normally consumed by the tissues and metabolism decreases. This stage in the animal response is shown in such experiments as 6, 14, 17, 19, 23, 43, 46 and 47.

The critical stages in the oxygen utilization coincide with the critical stages in the reactive mechanisms of the animal to progressive oxygen want. The blood oxygen crisis is limited to what Greene and Gilbert first designated the "post-crisis" in the respiratory and vascular responses to anoxemia.

Blood flow. The relation of the changes in the oxygen content of the blood to the circulatory responses is most marked when the blood flow is considered. Barcroft and his collaborators (2) have recently published a modification of the method of Zuntz whereby the minute volume may be calculated from the oxygen consumption and the oxygen content of the blood in the right and left ventricles respectively. A similar calculation of the blood flow may be made, using analyses of the arterial and jugular bloods. The oxygen content of the jugular blood is somewhat greater than that of the mixed venous blood, so that calculations of the apparent blood flow will be correspondingly high. The changes in this apparent blood flow probably accurately parallel the changes in the true blood flow.

The minute volume and the cardiac output in one series of experiments are shown in table 2. During the period of physiologic compensation there was little or no change in either the minute volume or cardiac output for each beat in dogs 6, 9, 10 and 11. The blood flow in dog 9 (table 2) was apparently constant and independent of the alveolar oxygen tension. The oxygen utilization from the blood was unchanged or slightly decreased in this animal which did not react with increased heart rate or sharp rise of the blood pressure at the approach of the crisis. Table 2 gives the blood pressure at the time of sampling only, and does not show the comparison with the initial pressures in these particular tests. Only experiment 33 progressed far enough to call forth some compensation. Here the initial heart rate was 99 and the blood pressure 83, both slightly under the sampling rate and pressure of the table. Minor variations in the oxygen utilization and in the blood flow were present as a result of changes in the blood pressure and the condition of the animal after several successive experiments. There was no significant change in the blood flow as a direct result of the anoxemia.

TABLE 1

Degree of saturation of venous blood in animals breathing air progressively reduced in oxygen tension

SERIES 1		INSPIRED AIR	VENOUS BLOOD					SERIES 2		ALVEOLAR AIR			VENOUS BLOOD			
Dog	Experiment	Oxygen tension	Oxygen capacity	Oxygen content	Saturation	Oxygen utilization	Utilization	Dog	Experiment	Oxygen tension	Carbon dioxide tension	Oxygen capacity	Oxygen content	Saturation	Oxygen utilization	Utilization
		mm.	*volumes per cent*	*volumes per cent*	*per cent*	*volumes per cent*	*per cent*			*mm.*	*mm.*	*volumes per cent*	*volumes per cent*	*per cent*	*volumes per cent*	*per cent*
1	1	159.3	25.1	14.1	56.0			7	25	85.4	29.5	16.30				
	2	21.1	25.1						26	34.7	20.5	16.30				
	3	159.3	25.1	16.2	64.5	8.0	31.9									
	4	22.3	25.1					8	27	133.0	21.1	11.70	7.85	67.1	3.60	30.8
									28	127.0	28.9	11.70				
2	5	159.3	16.6	11.4	68.6	1.5	9.1*		29	40.0	17.6	11.70	5.73	49.0*	0.33	2.8*
	6	20.5	16.6			0.0	0.0*		30	36.2	27.5	11.70				
	7	30.0	16.6													
	8	159.3	16.6					9	31	100.6	33.8	12.77	8.87	69.5	3.21	25.1
									32	64.0	24.9	12.77	7.14	55.9	3.33	27.0
3	9	159.3	13.1	7.5	57.2	4.2	32.1		33	27.9	19.2	12.77	0.33	2.6	3.33	26.1
	10	23.5	13.1						34	45.0	22.9	12.77	2.97	23.3	4.04	31.6
	11	159.3	13.1	7.5	57.2	2.9	22.1		35	89.2	26.7	12.77	3.40	26.6	4.76	37.3
	12	50.2	13.1						36	138.2	15.5	12.77				
									37	73.3	34.0	12.77				
4	13	159.3	22.9	12.1	52.8	9.8	42.6									
	14	14.9	22.9	0.4	11.8	1.8	8.0	10	38	92.0	34.9	15.44	10.63	68.8	3.35	21.8
	15	159.3	22.9	8.7	39.4*	12.1	54.6*		39	68.4	36.5	15.44	8.50	55.0	4.03	26.2
									40	43.5	36.0	15.44	7.78	50.4*	0.82	
5	16	159.3	23.7	18.1	76.4	4.6	19.4		41	34.8	36.0	15.44	5.70	36.9*	1.43	5.3*
	17	20.9	23.7	2.1	8.9	0.0	0.0*		42	28.6	39.6	15.44				9.2
	18	159.3	23.7	14.2	60.0	8.7	36.6		43	21.0	52.0	15.44	1.65	10.7*	0.00	0.0*
	19	36.6	23.7	3.4	14.3	0.0	0.0									

	20	159.3	23.7	13.0	54.8	9.0	38.2	11	44	59.7	24.5	7.88	2.80	35.5	2.40	30.5
									45	147.0	26.5	7.88				
6	21	159.3	24.5	18.4	73.9	4.8	19.2		46	35.3	14.5	7.88	0.89	11.3	0.03	0.4
	22	83.6	24.5	7.9	32.2	10.0	41.2*		47	14.6	14.8	7.88	0.20	2.5	0.63	8.0
	23	27.0	24.5	0.9	3.7	1.7	6.9									
	24	159.3	24.5	17.5	71.4	5.3	21.6	12	48	46.3	31.4	11.68	2.15	18.4		
									49	23.8	23.2	11.68				
									50	59.2	22.4	11.68				

* Because of the condition of the animal during the experiment or because of analytical discrepancies these experiments are felt to be less accurate than the remainder of the series. They are included in the table for the sake of completeness but are not shown in the figure.

TABLE 2

The oxygen utilization in the blood in animals breathing air progressively reduced in oxygen tension

ALVEOLAR AIR					BLOOD											
Dog	Experiment	Oxygen	Oxygen tension	Carbon dioxide tension	Oxygen capacity	Arterial content	Venous content	Oxygen utilization	Arterial saturation	Venous saturation	Utilization of hemoglobin	Total oxygen consumption each minute	Blood pressure	Blood flow, minute volume	Heart rate	Cardiac output, each beat
		per cent	*mm.*	*mm.*	*volumes per cent*	*volumes per cent*	*volumes per cent*	*volumes per cent*	*per cent*	*per cent*	*per cent*	*cc.*	*mm.*	*liters*		*cc.*
9	31	14.38	100.6	33.8	12.77	12.08	8.87	3.21	94.6	69.5	25.1	97.5	70	3.04	140	21.7
	32	8.61	64.0	24.9	12.77	10.47	7.14	3.33	82.9	55.9	27.0	97.5	90	2.92	120	24.3
	33	3.75	27.9	19.2	12.77	3.66	0.33	3.33	28.7	2.6	26.1	85.0	85	2.55	112	22.4
	34	6.06	45.0	22.9	12.77	7.01	2.97	4.04	54.9	23.3	31.6	87.5	68	2.17	118	19.0
	36	18.60	138.2	15.5	12.77	12.30			95.0				26			
11	47	1.90	14.6	14.8	7.88	0.83	0.20	0.63	10.5	2.5	8.0		129	16.60*	168	99.0*

* This calculation is based on the normal oxygen consumption of 105 cc. It represents the theoretical minute volume and cardiac output necessary to maintain physiologic activity at this arterial oxygen tension. The blood samples were taken just as respirations ceased and the oxygen consumption was approaching zero. The actual value obviously was much less than the theoretical.

These results are in accord with the findings of Doi (4), (5). Doi did not study the effects of oxygen tension lower than 100 mm., but down to this level the oxygen utilization and the minute volume were independent of the oxygen pressure. While the minute volume was constant the pulse rate increased with a correspondingly slight decrease in the cardiac output for each beat.

This condition is unchanged up to the point of the anoxemial crisis. With the exhaustion of the venous oxygen reserve the oxygen content of the arterial blood falls below the normal level of oxygen utilization, as in experiment 47. The normal blood flow in this animal was 2.91 liters each minute, the blood pressure was 82, the heart rate 129, and the cardiac output 22.6 cc. each beat. The blood samples were drawn for analysis just as respiration ceased and during the period of maximal blood pressure response.[1] The arterial blood sample in this experiment contained only 0.83 volumes per cent of oxygen. The maintenance of the normal rate of oxygen consumption during the anoxemial crisis would theoretically have required a blood flow of 16.6 liters each minute and a cardiac output of 99.0 cc. Cessation of respiration at the time the blood samples were taken is sufficient evidence that the circulation was unable to compensate for the extreme degree of oxygen want. Examination of the respiratory record shows that the oxygen consumption was greatly reduced during the half-minute preceding the actual stopping of respiratory movements. The narrow limits of circulatory compensation in this typical case should be emphasized. The maximal circulatory response results in only a four- to five-fold increase in the blood flow. This is in marked contrast to the large factor of safety present in other physiologic mechanisms, especially in such parenchymatous organs as the liver and kidneys.

General discussion. The correlation of the chemical changes in the blood and of the circulatory and respiratory responses of the organism indicates that these latter are entirely dependent on the disappearance of oxygen from the blood. In the normal animal and in the early stages of anoxemia, the arterial blood contains a large excess of oxygen over the requirement of the animal. There is an early though slight increase in the pulmonary ventilation and in the circulatory rate. These are slight, but add by so much to the efficiency of the gas exchange, in the lungs and in the tissues, a point presented in the intro-

[1] The blood pressure and respiratory record and the time of drawing samples in experiment 47 are shown in figure 2 of the preceding paper, Journ. Biol. Chem., 1922, lii, 143.

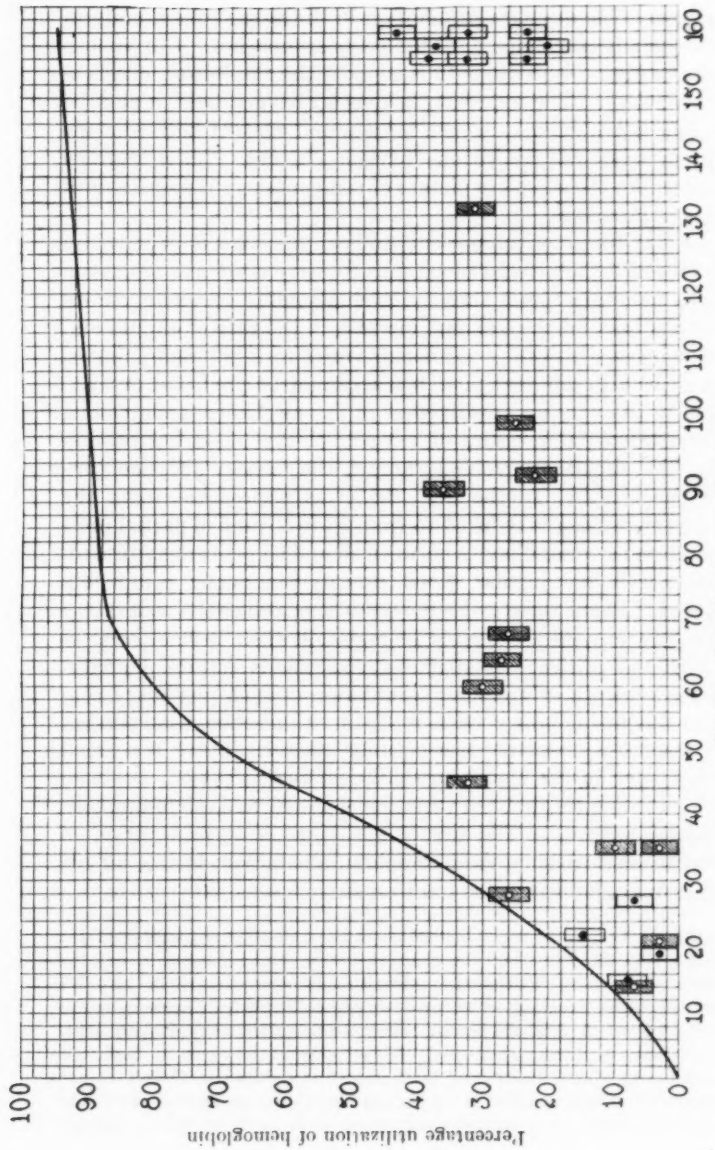

Fig. 3. Ordinates, percentage of utilization of hemoglobin. Abscissae, tension of oxygen in millimeters of mercury. Dissociation curve of dog's blood at 40 mm. tension of carbon dioxide. The cross-hatched rectangles denote determination plotted at oxygen tension of alveolar air. The plain rectangles denote determinations plotted at oxygen tension of inspired air. The area drawn around the point is the experimental error in each.

ductory pages. The oxygen consumption in the tissue runs a uniform course and there is no change until the crisis approaches. Our venous and arterial oxygen comparisons thus support the respiratory observations of Greene (6). The reduction in alveolar oxygen tension and the corresponding fall in arterial oxygen tension and content both coincide with a progressive fall in venous oxygen content. These parallel changes enter into the complete picture of compensatory responses until the crisis is approached.

From the standpoint of the present studies, this crisis is bounded by the point at which the arterial blood fails to carry a volume of oxygen adequate to maintain tissue activity, even assuming complete venous oxygen exhaustion and the maximal increase in the rate of circulation. The exact point at which the crisis occurs varies with different animals, and apparently with different men, hence cannot be predicted exactly. This is due to the varying sensitiveness of the mechanisms controlling *a*, the alveolar partial pressure of oxygen; *b*, the blood hemoglobin content; *c*, the circulatory volume; and *d*, the oxygen diffusion constants in the tissues. In the dog, the crisis approaches when the alveolar oxygen tension is reduced to from 25 to 35 mm. of partial pressure, or when the arterial blood is between 25 and 35 per cent saturated.

At these levels the jugular blood returns practically completely deoxygenated. Below these levels the cortical as well as the respiratory and circulatory centers are unable to maintain their normal level of activity. In this critical region unconsciousness is certain, notwithstanding the evidence of profound stimulation of such medullary centers as the cardio-inhibitory nucleus (9), a stimulation that persists after the respiratory activity has entirely ceased. There is a small content of oxygen in the blood during the post-crisis period, although the amount is within the limits of variation of the current micro-methods used in blood analyses. The tissues withstand a certain period of complete anoxemia without their complete destruction, but of all tissues the nervous centers are the most sensitive.[2]

[2] While this paper was in press, an article was published by Morris (Journ. Physiol., 1922, lvi, 283) reporting the changes in the blood oxygen in cats as tested by five rebreathing experiments. Morris made no analysis of the alveolar air and gave no blood pressures or other adequate evidence for determining the change in the volume flow of the blood. Morris reports that the oxygen utilization is not constant during anoxemia. His conclusion, however, is based on two experiments in which the arterial saturation was reduced to 17 and 19 per cent respectively. The saturation of the jugular blood in these same experiments was 22 and 21 per cent. A percentage saturation of 21 to 22 per cent in jugular

SUMMARY

In the intact animal we have supported by new analytical and experimental data the following points:

1. The utilization of oxygen from the blood is nearly constant, and is independent of the arterial oxygen tension until the venous oxygen reserve is exhausted. Thereafter, the oxygen utilization parallels the saturation of the arterial blood.

2. Respiratory and circulatory crises occur at the approach of the point where the arterial saturation is reduced below the normal level of oxygen utilization.

3. The oxygen tension of the alveolar air does not materially affect the blood flow until the crisis is approached.

4. The blood flow and the oxygen utilization may show minor changes secondary to changes in the blood pressure or to the condition of the animal. In such changes the blood flow and the oxygen utilization vary inversely.

5. The venous oxygen content represents an oxygen reserve available, apart from the circulatory compensations, for an increased oxygen consumption above the basal level.

blood is a considerable oxygen reserve in the sample. This reserve is too great to produce either respiratory or vascular crises in dogs certainly, and cats probably. A simultaneous arterial lower saturation is not possible at this level under any normal condition in dogs. We have emphasized the constancy in oxygen utilization in the precrisis stage of anoxemia and the first three experiments of Morris are in harmony with our conclusions.

Morris also emphasized the difference between the oxygen content of the blood in the jugular veins and the right heart. In the two experiments he publishes the jugular blood was slightly more saturated than the arterial blood. He states that this difference is probably to be accounted for by the oxygen utilization by the pulmonary tissues. This conclusion assumes a perfect balance as between the venous blood and the alveolar air and does not take into account the possible source in the alveolar air of any oxygen used by the pulmonary tissues. The experiments reported by Morris imply a much higher percentage deviation in the method than that obtainable by the Van Slyke method. Because of the technical errors inherent in blood gas analysis and in view of the absence of coincident determination of alveolar air and blood vascular measurements by Morris, we are unable to agree with his conclusions as regards the variations in oxygen utilization in asphyxia.

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REPRODUCTION ON "SYNTHETIC" DIETS WHEN PURIFIED AGAR IS ADDED TO THE MIXTURE

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According to the report of Evans and Bishop (1) on the relations between fertility and nutrition, there seems to be nothing about a standard nutritional regime which is inconsistent with normal growth and reproduction. However, our failure to secure reproduction in rats on the Osborne and Mendel (2) "standard casein diet" plus yeast accords with the experience of the latter and with that of several other investigators. This diet consists of casein, 18; starch, 51; salt mixture, 4 (3); butter fat, 9; crisco, 18 per cent respectively with 0.4 gram of dry yeast daily as a source of vitamin B. The rats grow to large size and apparently splendid condition on such a "synthetic" diet but frequently will not breed. In the few cases of our experience where the females did become pregnant they either died just before the litter should have been born or the young were dead or died soon after birth in almost every instance. Autopsies were performed on all females which died during pregnancy or following delivery and in practically every case there appeared to be a severe intestinal obstruction. Although it has been assumed by many workers that rats can thrive on diets free from roughage, nevertheless it seemed that bulk might be a beneficial factor especially in the diet of the pregnant female. Following the suggestion offered by this observation, 5 per cent of purified agar was substituted for an equal weight of starch and incorporated in the food mixture which otherwise was unchanged.

Two females which had been on the "standard casein diet" for 14 and 25 weeks respectively and had been mated without result were again mated with the same male after agar had been added to the food and the following month produced and raised litters of seven and ten respectively. Two younger females which had been raised on the "standard" with agar have each produced a normal, healthy litter.

Some other laboratories have reported that by the addition of certain natural foods to the "synthetic" diets they have succeeded in securing

reproduction. It has not been determined just what factor in these natural foods is responsible for the beneficial effect observed. Since the agar used in these experiments was a highly purified product containing only slight traces of salts which were already present in the food in abundance, it does not seem that these substances could have any significant bearing on the subject. Protein (N × 6.25) determined by the Kjeldahl method was found to be 1.8 per cent of the agar. It has not been established that any of this nitrogen is available to the animal organism but even if it were all utilized the proportion present in the food would be only 0.09 per cent, an amount which would seem to be entirely insignificant as a supplementary protein.

From these preliminary observations we conclude that roughage, as such, in the diet of the rat may be a far more important factor than has previously been supposed. More extensive investigations are being carried on along this line, the details of which we shall hope to report in the near future.

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THE EFFECTS OF INSULIN ON EXPERIMENTAL HYPERGLYCEMIA IN RABBITS

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In a previous paper it was shown that a marked fall occurs in the percentage of blood sugar in normal rabbits when they are injected subcutaneously with insulin. Taken in conjunction with the fact that insulin also reduces often to the normal level, or even below it, the high percentages of sugar found in the blood of depancreated dogs and of diabetic patients, it would appear that its action must be a fundamental one in the control of the blood sugar level (1). The present investigation was undertaken to obtain further evidence of the scope of the action of insulin by studying its effect on the various experimental conditions that are known to cause marked hyperglycemia in rabbits.

It is unnecessary to review here the extensive literature which bears on the methods used to cause these various forms of hyperglycemia. We will only refer to those investigations which have a direct bearing on our own results, in connection with each of the varieties which we have investigated.

METHODS. The rabbits used were as uniform in size and breed as possible and they were fed for some days preceding the experiments an abundance of oats and hay, sometimes with sugar added. The blood was collected at frequent intervals in 1 cc. quantities from the ear veins and the percentage of sugar determined in the samples by the Schaffer-Hartman method. At the termination of the experiments, whenever possible, the percentage of glycogen was determined in the liver by Pflüger's method, using the Schaffer-Hartman method for measurement of the reducing power of the hydrolyzed solutions. The insulin used was not always of uniform potency since during the progress of this research we were also engaged in working out the most suitable method for its preparation. As a preliminary to each experi-

ment it was therefore the practice to inject the preparation of insulin into normal rabbits. Provided the preparation was found to be active, either the same rabbit or another (normal) rabbit was subjected to one or other of the procedures for the production of hyperglycemia. These were piqûre, injection of epinephrin, mechanical asphyxia, carbon monoxide poisoning and ether.

RESULTS: *Piqûre.* In order to be certain that hyperglycemia will result in this experiment it is necessary to make sure that the liver contains adequate amounts of glycogen and that the puncture is not too far above the calamus of the medulla and is near the midline.

With regard to the latter condition it is our opinion that certainty of correct puncture can best be assured by reflecting the skin from the occipital bone under local (ethyl chloride) anesthesia and then, with the head bent as far forward as possible, puncturing at the occipital tubercle in the direction of the outer canthi of the eyes until the point of the instrument is felt to come against the basilar process. We have found that an ordinary trochar is a suitable instrument to use. This operation necessarily wounds the cerebellum with the result that after it the animal shows forced movements. Usually also there is a certain amount of hemorrhage into the 4th ventricle. Stewart and Rogoff (2) recommend actual exposure of the 4th ventricle by Eckhard's method, which involves separating the muscles lying over the occipito-atlantoid ligament, which is then incised. We believe however that this is unnecessary and that it is an advantage to avoid it because of the danger of hemorrhage. At the termination of each experiment the exact position of the puncture was determined by post-mortem examination.

The results of piqûre on three normal well-fed rabbits were as follows:

1. Rabbit II, 16; weight, 2.3 kilos

No normal blood sample taken.
Piqûre at 11:25 a.m.
Blood at 11:35—0.151 per cent sugar.
Blood at 12:05—0.282 per cent sugar.
Blood at 12:35—0.350 per cent sugar.
Blood at 2:50—0.387 per cent sugar.
Blood at 3:35—0.324 per cent sugar.
Blood at 4:00—0.280 per cent sugar.
Blood at 5:00—0.170 per cent sugar.
Blood at 5:45—0.161 per cent sugar.

Glycogen determination not made. Post mortem of the medulla showed two punctures (see fig. 2), one 2 mm. to the right of the midline, 3 mm. above the calamus scriptorius, and the other 2 mm. to the left, 8 mm. above the calamus.

2. Rabbit III, ♂

Normal blood at 10:15 a.m.—0.137 per cent sugar.
Piqûre at 11:00.
Blood at 11:30—0.305 per cent sugar.
Blood at 12:05—0.420 per cent sugar.
Blood at 12:50—0.457 per cent sugar.
Blood at 2:20—0.386 per cent sugar.
Blood at 3:20—0.244 per cent sugar.
Blood at 4:25—0.187 per cent sugar.
Blood at 12:30—0.170 per cent sugar (next day).
Animal killed at 12:30 next day.

The liver at this time showed a glycogen content of 2.0 per cent. Post mortem showed a puncture through the vermis of the cerebellum in the midline, and through the medulla 1 mm. to the left of the midline, 11 mm. above the calamus.

3. Rabbit IV, 1; weight, 2.15 kilos.

Normal blood at 9:15 a.m.—0.174 per cent sugar.
Piqûre at 10:45 a.m.
Blood at 11:30—0.370 per cent sugar.
Blood at 12:00—0.390 per cent sugar.
Blood at 1:00—0.380 per cent sugar.
Blood at 2:00—0.400 per cent sugar (boiled too long, therefore too high).
Blood at 4:00—0.330 per cent sugar.
Blood at 4:30—0.317 per cent sugar.
Blood at 8:30—0.203 per cent sugar.
Animal killed at 3:30 p.m. next day. Liver showed a glycogen content of 0.59 per cent. The puncture of the medulla was oblique, 1 mm. to the right of the midline and extending from 8 to 15 mm. above the calamus scriptorius.

These results are given in curve form (fig. 1) along with two of Stewart and Rogoff's and the position of the punctures is shown in figure 2. It will be seen that the rise in blood sugar follows the piqûre very rapidly indeed, the maximum being reached in about one hour, and that the return to normal is much slower occupying from 5 to 8 hours. The glycogen content of the rabbit showing the steepest curve (no. 2) was found to be 2 per cent on the day following the experiment. It was lower, 0.6 per cent, in another (no. 3) animal in which the curve was somewhat lower. The considerable variability in the position of the punctures in these three definitely positive cases of hyperglycemia shows that some latitude is permissible.

Turning now to the experiments in which the piqûre was performed on animals under the influence of insulin very different results are evident.

4. Rabbit IV, 2

Normal blood at 12:50—0.123 per cent sugar.
At 3:30, 5 cc. of insulin.
Blood at 4:30—0.083 per cent sugar.
Piqûre at 4:45.
Blood at 5:15—0.081 per cent sugar.

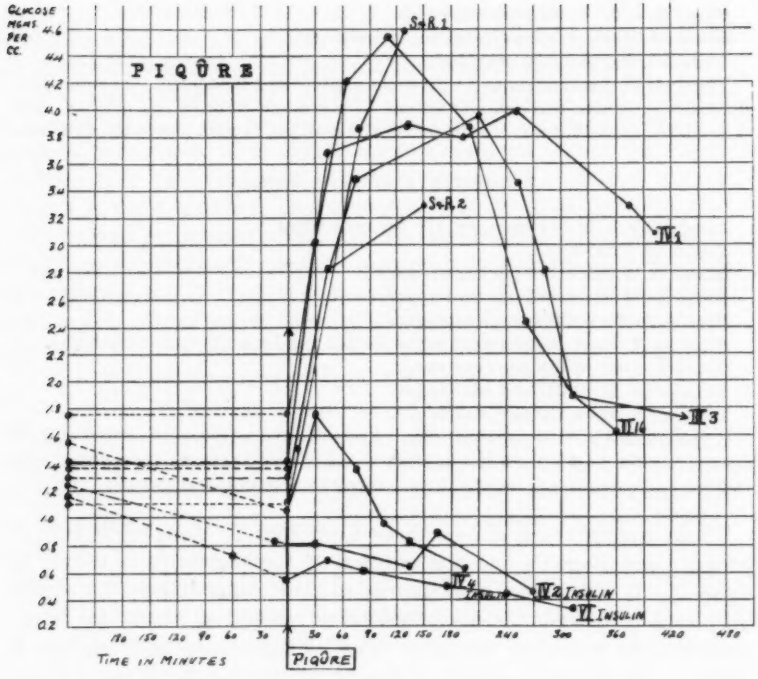

Fig. 1

At 5:20 4 cc. of insulin.
Blood at 6:00—0.064 per cent sugar.
Blood at 6:30—0.093 per cent sugar.
Blood at 8:13—0.045 per cent sugar.
Blood at 3:00—0.124 per cent sugar (next day).

At 8:15 the rabbit was in convulsions as described in the previous paper and dextrose solution was injected subcutaneously, resulting in rapid recovery. The rabbit was killed at 3:30 the next day, and the liver showed only 0.27 per cent

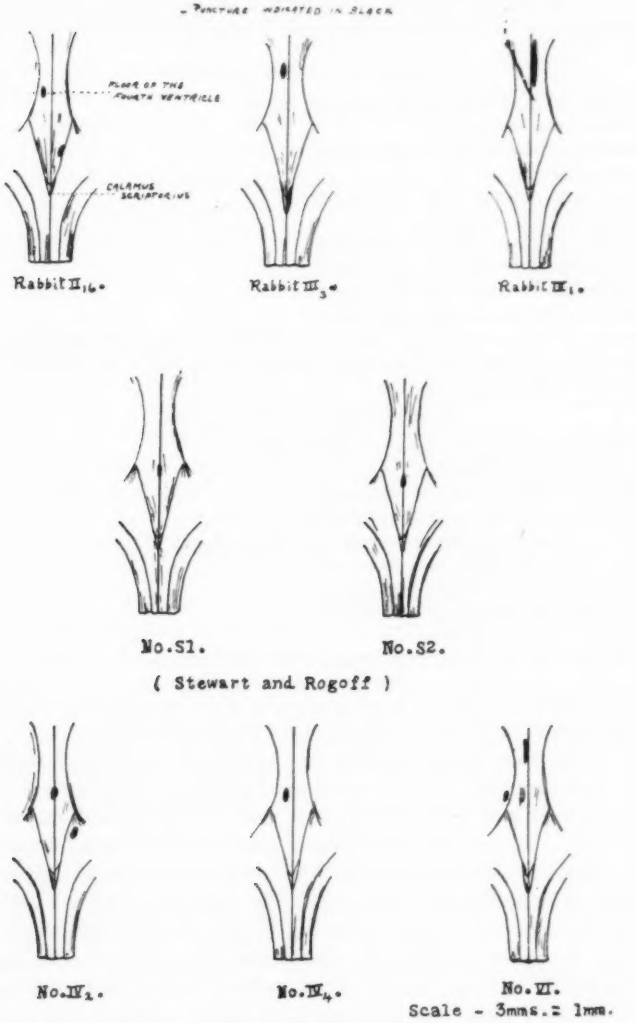

Fig. 2. Diagram indicating the seat of puncture in the floor of IV ventricle.

glycogen. The medulla showed two punctures, one on the right cerebral peduncle, 6 mm. above the calamus, and the other 10 mm. above in the midline (no. 4 in fig. 2).

5. Rabbit IV, 4; weight 1.8 kilos

Normal blood at 9:25—0.158 per cent sugar.
At 10:35, 4 cc. of insulin.
Blood at 11:15—0.106 per cent sugar.
Piqûre at 11:30.
Blood at 12:00—0.177 per cent sugar
At 12:05, 3 cc. of insulin.
Blood at 12:45—0.138 per cent sugar
Blood at 1:15—0.096 per cent sugar.
Blood at 1:45—0.085 per cent sugar.
Blood at 2:45—0.063 per cent sugar.
Animal killed at 3:15 p.m. Liver contained 4.4 per cent glycogen. Post mortem showed a puncture of the medulla 2 mm. to the left of the midline, 10 mm. above the calamus (fig. 2, no. 5).

6. Rabbit VI; weight, 2.4 kilos

Normal blood at 9:50—0.117 per cent sugar.
At 10:00—7 cc. of insulin.
Blood at 11:00—0.075 per cent sugar.
Blood at 11:45—0.056 per cent sugar.
Piqûre 11:50 (2 punctures) about 5 cc. of blood lost.
Blood at 12:35—0.068 per cent sugar.
Blood at 1:10—0.062 per cent sugar.
Blood at 2:40—0.052 per cent sugar.
At 3:00—3 cc. of insulin.
Blood at 3:45—0.042 per cent sugar, mild convulsions.
Blood at 4:55—0.035 per cent sugar, mild convulsions.
Animal killed at 5:00. Liver contained 2.64 per cent glycogen. Puncture through vermis in midline, two in floor of 4th ventricle (fig. 2, no. 6).

The punctures of the medulla in these three cases were such as to insure hyperglycemia. The amounts of glycogen found present in the livers were entirely adequate in two cases (5 and 6) but rather small in one (4). In this case the animal was not killed until twenty-two hours after the operation, during which time a considerable amount of glycogen may have been hydrolyzed. In experiment 4 the blood sugar during the first four hours following piqûre did not rise above 0.093 per cent, a figure considerably under the normal level. In no. 5, it will be noted that the sugar was not reduced to the same extent as in no. 6, before piqûre was performed; in thirty minutes it had risen to 0.177 per cent slightly above normal, and then, following another dose of extract, fell off gradually to 0.063 per cent. (These results are shown in graphic form in fig. 1.)

From these experiments, we may conclude that the severe hyperglycemia which occurs in rabbits whose livers are rich in glycogen, following piqûre, may be markedly reduced, if not entirely inhibited, if the operation is performed subsequent to the injection of suitable doses of insulin. The experiment, no. IV, 2, in which 0.27 per cent of glycogen was found in the liver, is the only one in which there can be any doubt as to the inhibiting action of insulin. It is somewhat similar to one described by Stewart and Rogoff (*loc. cit.*) in which no hyperglycemia occurred in a piqûred rabbit with 0.34 per cent of glycogen in the liver. In this case the blood sugar rose only from 0.124 per cent to 0.143 per cent following piqûre and to 0.151 per cent following asphyxia. In our case, as a result of insulin, the blood sugar fell to 0.045 per cent and then recovered to 0.124 per cent by next day. This recovery probably indicates that the glycogen had been drawn on to restore the blood sugar to its normal level. In light of the corroborative nature of the results of other experiments on piqûre we do not consider it necessary at the present to add further observations.

Epinephrin hyperglycemia. Bang (3) found that the subcutaneous injection of 1 mgm. epinephrin in rabbits caused the blood sugar to rise to a maximum in 2 to 3 hours returning to the normal in 7 to 9 hours. The curve was similar in starved and well-fed animals except that it did not begin to rise quite so quickly in the former. Reference to other investigations are given by Bang, the most significant point being that injection intravenously of the above amount of epinephrin causes only a transient and slight increase in blood sugar.

In order to satisfy ourselves that marked hyperglycemia invariably follows subcutaneous injection of epinephrin in rabbits, the following experiments were done using solutions of adrenalin chloride.

1. Rabbit IV, 3; weight 1.45 kilos; well-fed

Normal blood at 9:15—0.154 per cent sugar.
At 10:20, 2 cc. adrenalin chloride solution 1—1000 injected subcutaneously.
Blood at 10:55—0.364 per cent sugar.
Blood at 11:30—0.397 per cent sugar.
Blood at 12:00—0.440 per cent sugar.
Blood at 1:00—0.440 per cent sugar.
Blood at 2:05—0.410 per cent sugar.

2. Rabbit II, 6; well-fed

Normal blood at 10:50—0.141 per cent sugar.
At 2:35, 2 cc. adrenalin chloride solution 1—1000 injected subcutaneously.
Blood at 3:10—0.34 per cent sugar.

Blood at 4:55—0.36 per cent sugar.
This is the same adrenalin solution as used in the insulin exper. (III, 6).

3. Rabbit III, 2

Normal blood at 9:55—0.120 per cent sugar.
At 9:58, 2.0 cc. adrenalin chloride 1—1000, subcutaneously.
Blood at 10:35—0.291 per cent sugar.
Blood at 11:05—0.340 per cent sugar.
Blood at 11:35—0.390 per cent sugar.
Blood at 12:20—0.416 per cent sugar.
Blood at 1:15—0.391 per cent sugar.
Blood at 2:15—0.349 per cent sugar.
Blood at 3:15—0.317 per cent sugar.

4. Rabbit III, 3

Normal blood at 9:35—0.124 per cent sugar.
At 9:47, 1.0 cc. adrenalin chloride.
Blood at 10:17—0.195 per cent sugar.
Blood at 10:47—0.296 per cent sugar.
Blood at 11:25—0.340 per cent sugar.
Blood at 12:30—0.369 per cent sugar.
Blood at 1:30—0.364 per cent sugar.
Blood at 2:30—0.354 per cent sugar.

5. Rabbit IV, 6; weight 1.65 kilos; sugar-fed

Normal blood at 9:50—0.117 per cent sugar.
At 9:55, 1.0 cc. of adrenalin chloride 1—1000[1] injected subcutaneously.
Blood at 10:25—0.223 per cent sugar.
Blood at 10:55—0.270 per cent sugar.
Blood at 11:55—0.273 per cent sugar.
Blood at 1:10—0.158 per cent sugar.

To study the influence of insulin on this form of hyperglycemia, two methods were used. In the following two experiments 2 cc. adrenalin and 5 cc. of insulin were injected at the same time.

1. Rabbit III, 6; well-fed

Normal blood at 12:00—0.122 per cent sugar.
At 2:45, 5 cc. of insulin and 2.0 cc. adrenalin chloride 1—1000 were injected subcutaneously.
Blood at 3:30—0.090 per cent sugar.
Blood at 4:15—0.132 per cent sugar.
Animal died at 5:40 p.m. No glycogen determination made.

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[1] This is the same adrenalin solution as used in the insulin experiments IV, 5 and IV, 6.

2. Rabbit X

Normal blood at 9:50—0.138 per cent sugar.
At 9:56, 4.0 cc. insulin + 2 cc. adrenalin chloride 1—1000.
Blood at 10:30—0.232 per cent sugar.
Blood at 11:00—0.303 per cent sugar.
Blood at 11:30—0.347 per cent sugar.
Blood at 12:10—0.365 per cent sugar.
Blood at 1:10—0.388 per cent sugar.
Blood at 2:10—0.325 per cent sugar.
Blood at 3:10—0.233 per cent sugar.
Blood at 4:10—0.165 per cent sugar.
This insulin caused convulsions in a control animal.

In the following three experiments 2 cc. adrenalin were injected after the hypoglycemic effect of insulin had become evident.

1. Rabbit VII, weight, 4.25 kilos

Normal blood at 10:15—0.127 per cent sugar.
At 10:17, 4 cc. insulin.
Blood at 11:15—0.106 per cent sugar.
2 cc. adrenalin chloride 1–1000, subcutaneously.
Blood at 11:45—0.183 per cent sugar.
Blood at 12:15—0.227 per cent sugar.
Blood at 12:45—0.240 per cent sugar.
Blood at 1:15—0.244 per cent sugar.
Blood at 1:45—0.307 per cent sugar.
Blood at 2:15—0.324 per cent sugar.
Blood at 2:45—0.304 per cent sugar.
Blood at 3:45—0.235 per cent sugar.
Blood at 4:45—0.147 per cent sugar.
Blood at 6:30—0.115 per cent sugar.

2. Rabbit VIII

Normal blood at 11:00—0.122 per cent sugar.
At 12:15, 2.0 cc. insulin.
Blood at 2:15—0.095 per cent sugar.
Blood at 4:00—0.067 per cent sugar.
Blood at 4:30—0.065 per cent sugar.
At 4:35, 2.0 cc. adrenalin chloride.
Blood at 5:05—0.136 per cent sugar.
Blood at 6:00—0.182 per cent sugar.
Blood at 6:55—0.269 per cent sugar.
Blood at 7:25—0.321 per cent sugar.

3. Rabbit IX

Normal blood at 11:00—0.125 per cent sugar; 2.0 cc. insulin.
Blood at 2:15—0.085 per cent sugar.

Blood at 4:00—0.069 per cent sugar.
Blood at 4:30—0.068 per cent sugar.
At 4:35, 2.0 cc. adrenalin chloride.
Blood at 5:10—0.110.
Could not obtain blood.

In the following five experiments 1 cc. adrenalin was injected after the hypoglycemic effect of insulin had become evident.

1. Rabbit XII

Normal blood at 9:05—0.105 per cent sugar.
At 9:10, 2.0 cc. insulin.
Blood at 11:15—0.083 per cent sugar.
Blood at 12:15—0.050 per cent sugar.
1.0 cc. adrenalin chloride 1–1000.
Blood at 1:15—0.054 per cent sugar.
Blood at 2:15—0.090 per cent sugar.
Blood at 3:20—0.090 per cent sugar.
Blood at 4:15—0.090 per cent sugar.
Glycogen W_3 = 2.26 per cent.

2. Rabbit XIII

Normal blood at 9:25—0.100 per cent sugar.
At 9:30, 2.0 cc. insulin.
Blood at 11:25—0.066 per cent sugar.
Blood at 1:10—0.074 per cent sugar.
1.0 cc. adrenalin chloride 1–1000.
Blood at 2:10—0.120 per cent sugar.
Blood at 3:15—0.131 per cent sugar.
Blood at 4:10—0.127 per cent sugar.
Glycogen B_3 = 1.80 per cent.

3. Rabbit XIV

Normal blood at 10:10—0.130 per cent sugar.
At 10:12, 3 cc. insulin.
Blood at 12:10—0.099 per cent sugar.
Blood at 1:25—0.092 per cent sugar.
1 cc. adrenalin subcutaneously.
Blood at 2:25—0.250 per cent sugar.
Blood at 3:25—0.265 per cent sugar.
Blood at 4:25—0.248 per cent sugar.

4. Rabbit IV, 7; well-fed

No normal blood sample obtained.
At 5:00, 10 cc. of insulin injected subcutaneously.
Blood at 6:00—0.042 per cent sugar (rabbit in convulsions).
At 6:15, 1.0 cc. adrenalin chloride 1–1000 subcutaneously.
Blood at 7:20—0.030 per cent sugar.

Blood at 8:05—0.035 per cent sugar.
Blood at 8:55—0.060 per cent sugar.
Blood at 10:45—0.056 per cent sugar (taken from heart).
Animal killed at 10:45. Liver contained 0.77 per cent of glycogen. Throughout this experiment the animal was subject to convulsions of from one to two minutes duration at about fifteen-minute intervals, between which its condition seemed to be considerably improved.

5. Rabbit XI

Normal blood at 9:00—0.111 per cent sugar.
At 9:30, 4.0 cc. insulin subcutaneously.
Blood at 11:35—0.090 per cent sugar.
At 11:40—1 cc. adrenalin chloride.
Blood at 12:30—0.166 per cent sugar.
Blood at 11:40—0.180 per cent sugar
Blood at 1:30—0.188 per cent sugar } Obtained from heart.
Blood at 2:30—0.176 per cent sugar

It is clear that insulin is capable of greatly reducing the hyperglycemia caused by epinephrin, provided the latter be not given in massive doses. Out of a total of five experiments in which 1 cc. adrenalin chloride was injected after the hypoglycemic effect of insulin had become evident, the blood sugar did not rise above 0.130 per cent in three, in one it rose to 0.190 and in another to 0.265. In the two last mentioned cases however the insulin used was evidently extremely weak, the blood sugar being only reduced to about 0.09 per cent prior to the injection of adrenalin, thus contrasting with the marked reduction in the other cases. In the three experiments in which 2 cc. adrenalin were injected following insulin a decided hypoglycemic effect remained in one, but distinct increase in blood sugar occurred in the other two. In two experiments in which 2 cc. adrenalin were injected at the same time as insulin, a marked hyperglycemia developed in the one but not in the other. Taking these results as a whole it is plain that much more work must be done before it can be told to what extent insulin can antidote the adrenalin effect. We are hopeful that it may be possible to determine the dosage of insulin in terms of the amount capable of antidoting the effect of a standard dose of adrenalin. These results are given in curve form in figures 3 and 3a.

Asphyxial hyperglycemia. There is no more certain means for causing a marked degree of hyperglycemia than asphyxia brought about by constriction of the upper air passages. In rabbits the most practical way for doing this is by placing a piece of water-proof material over the

snout and holding it there until the heart rate becomes definitely slowed. The animal is then allowed to breathe freely for a few breaths when it is again asphyxiated. This procedure is kept up for twenty minutes. With so many observations of this type on record (cf. Stewart and

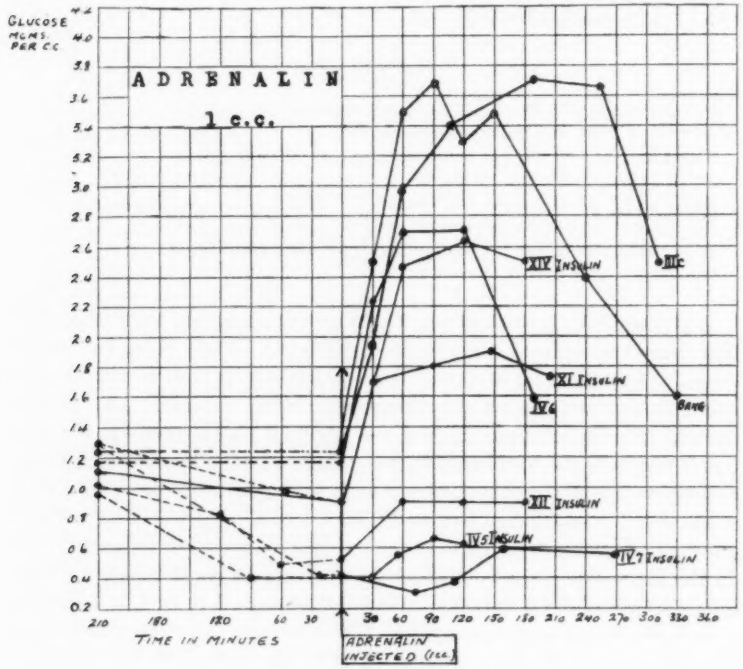

Fig. 3a

Rogoff, and Macleod) it was not considered necessary to perform more than one control experiment of which the following is the result.

1. Rabbit IV, ♂; weight 2.3 kilos; well-fed

Normal blood at 9:50—0.140 per cent sugar.
Mechanical asphyxia from 9:55–10:20.
Blood at 10:23—0.383 per cent sugar.
Blood at 10:55—0.376 per cent sugar.
Blood at 11:25—0.370 per cent sugar.
Blood at 11:55—0.222 per cent sugar.
Blood at 12:25—0.200 per cent sugar.

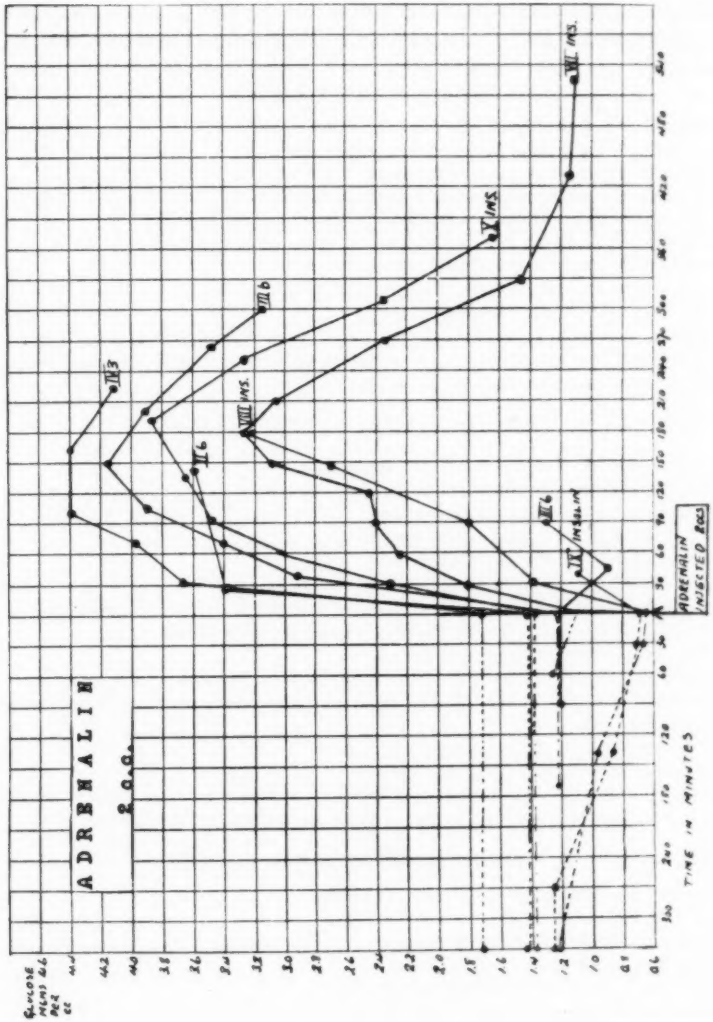
ADRENALIN
2 c.c.c.
III 3
II 6
VIII b
VIII ins.
X ins.
XII ins.
XI 6
XI 6 Insulin
ADRENALIN INJECTED
TIME IN MINUTES

Fig. 3b

Three experiments were then performed on well-fed rabbits which were asphyxiated for twenty minutes following the injection of insulin.

2. Rabbit IV, 4; weight 2.05 kilos

Normal blood at 9:50—0.124 per cent sugar.
At 10:00, 3 cc. insulin injected subcutaneously.
Blood at 11:00—0.077 per cent sugar.
At 2:00, 3.0 cc. insulin (same) subcutaneously.
Blood at 2:20—0.045 per cent sugar (animal hyperexcitable).
Mechanical asphyxia from 2:22–2:40.
Blood at 2:45—0.159 per cent sugar.
Blood at 3:15—0.075 per cent sugar.
Blood at 3:45—0.046 per cent sugar.
Animal was asphyxiated again, and died during the operation.
Blood at 4:15—0.144 per cent sugar obtained from the heart after death.
Liver contained 2.04 per cent glycogen.

3. Rabbit IV, 9; weight, 2.3 kilos; sugar-fed

Normal blood at 10:00—0.117 per cent sugar.
At 10:05, 4.5 cc. insulin injected subcutaneously.
Blood at 11:45—0.083 per cent sugar.
At 12:40, 4.0 cc. insulin subcutaneously.
Blood at 2:00—0.079 per cent sugar.
At 2:55, 12.0 cc. insulin (same) subcutaneously.
At 3:10 the animal was in convulsions; it was asphyxiated from 3:15 to 3:35.
Blood at 3:40—0.075 per cent sugar; animal hopping about.
Blood at 4:20—0.090 per cent sugar.
Blood at 4:50—0.035 per cent sugar; animal in convulsions.
Mechanical asphyxia again from 4:55–5:15, animal seemed to be considerably recovered.
Blood at 5:20—0.077 per cent sugar.
Blood at 5:55—0.039 per cent sugar.
At 6:10 animal was in violent convulsions. Killed at 6:15. Blood from the heart at 6:15—0.062 per cent sugar. Glycogen in the liver, 2.69 per cent.

4. Rabbit IV, 10; weight 2.65 kilos; sugar-fed

Normal blood at 9:40—0.139 per cent sugar.
At 9:41,5.0 cc. of insulin subcutaneously.
Blood at 10:25—0.075 per cent sugar.
Blood at 11:15—0.062 per cent sugar.
At 11:25, 6.0 cc. of insulin subcutaneously.
Mechanical asphyxia from 11:30—11:50.
Blood at 11:50—0.075 per cent sugar.
Blood at 12:20—0.064 per cent sugar.
Blood at 12:50—0.056 per cent sugar.
Animal killed at 1:00. Liver contained 5.3 per cent glycogen.

These results are shown in curve form in figure 4 and it will be seen that insulin had the effect either of preventing entirely any asphyxial rise in blood sugar or of greatly reducing the rise which usually occurs. In Experiment IV, 4, the sugar rose from 0.045 to 0.159 per cent during a twenty-minute asphyxial period, an increase of 0.114 per cent as com-

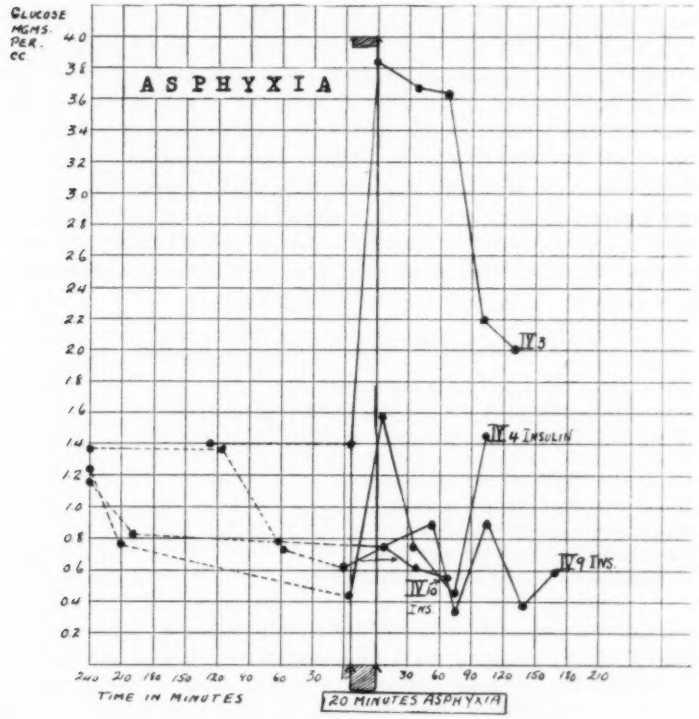

Fig. 4

pared with 0.243 per cent in the control experiment (no. IV, 3) but it will be observed this rise was of a very temporary nature, the percentage returning to its pre-asphyxial level in one hour. A similar sharp return to the pre-asphyxial level is also to be observed in the second period of asphyxia in experiment IV, 9.

Carbon monoxide poisoning. In order to study the effect of a less acute form of asphyxia than the foregoing we have also investigated

carbon monoxide poisoning. The method was to place the animal in an air-tight box just large enough to contain it comfortably, and provided with an observation window. A mixture of air and illuminating gas was then allowed to circulate slowly through the box at the rate of 0.6 liter per minute. This mixture contained 0.8 per cent carbon monoxide by calculation. It was probably considerably less than this since Haggard and Henderson found that 0.4 per cent was sufficient. In the 0.8 per cent atmosphere hyperpnea developed early and in forty-five minutes the animal was in a semi-conscious condition and was removed from the box. It was then unable for several minutes to stand on its feet and some time elapsed before the hyperpnea disappeared. Four of the five experiments performed were carried out on two well-fed rabbits, each of which was first subjected to forty-five minutes' gassing, and then in a week's time, underwent a second period of gassing, after being given insulin. The fifth experiment is an additional control performed on a normal rabbit. The results of the five experiments are as follows:

1. (a) Rabbit III, 10; weight, 2.2 kilos; well-fed

Normal blood at 2:30—0.149 per cent sugar.
Gassed from 2:30–3:18, 0.8 per cent CO (by calculation).
Blood at 3:19—0.245 per cent sugar.
Blood at 3:49—0.334 per cent sugar.
Blood at 4:19—0.347 per cent sugar.
Blood at 4:49—0.320 per cent sugar.
Respirations at 3:20—240 per minute
Respirations at 3:45—250 per minute.
Respirations at 4:30—190 per minute.

(b) Same animal seven days later

Normal blood at 10:00—0.125 per cent sugar.
At 10:05, 3.0 cc. of insulin, subcutaneously.
Blood at 10:40—0.089 per cent sugar.
At 10:45, 5.0 cc. insulin, subcutaneously.
Gassed from 10:52–11:37, 0.8 per cent CO.
Blood at 11:40—0.081 per cent sugar.
Blood at 12:10—0.083 per cent sugar.
At 12:15, 2.5 cc. insulin subcutaneously.
Blood at 12:40—0.070 per cent sugar.
Blood at 1:10—0.060 per cent sugar.
Blood at 2:30—0.070 per cent sugar.
Blood at 3:30—0.062 per cent sugar.
Animal was in very weak condition when removed from the chamber.

2. (a) Rabbit III, 11; weight 2.4 kilos; well-fed

Normal blood at 1:30—0.137 per cent sugar.
Gassed from 1:37–2:02 (only twenty-five minutes).
Blood at 2:03—0.211 per cent sugar.
Blood at 2:33—0.219 per cent sugar.
Blood at 3:03—0.203 per cent sugar.
Blood at 3:33—0.171 per cent sugar.
Blood at 9:30—0.136 per cent sugar (next day)..
Animal removed in twenty-five minutes because it seemed to be in an unconscious condition.

(b) Same rabbit three days later

Normal blood at 2:45—0.135 per cent sugar.
At 2:50, 5.0 cc. insulin subcutaneously.
Gassed 3:02–3:46, 0.8 per cent CO (by calculation).
Blood at 3:46—0.143 per cent sugar.
Blood at 4:16—0.150 per cent sugar.
Blood at 4:46—0.127 per cent sugar.
Blood at 5:16—0.095 per cent sugar.
Animal was in a very weak condition when removed from the chamber.

3. Rabbit III, 12; weight, 2.3 kilos

Normal blood at 9:40—0.119 per cent sugar.
Gassed from 9:45–10:29, 0.8 per cent CO.
Blood at 10:30—0.286 per cent sugar.
Blood at 11:06—0.257 per cent sugar.
Blood at 11:30—0.145 per cent sugar.
Blood at 12:00—0.129 per cent sugar.
Animal seemed to recover rapidly after removal from the chamber.

In experiment 1 (b) the results are very striking; the blood sugar immediately after the animal was removed from the chamber, was slightly lower than when the animal was inserted forty-four minutes before. The same rabbit seven days previously had responded to the same period of gassing, without the injection of insulin, by a rise in blood sugar to as high as 0.347 per cent. In experiment 2 (b) the sugar rose very slightly (from 0.135 to 0.150 per cent) when subjected to carbon monoxide after administration of insulin, but in this case, the insulin was given only twelve minutes before the animal was placed in the chamber, so that the full force of its reducing powers might not have come into effect. In the control experiment, performed previously on the same rabbit, the blood sugar rose to 0.22 per cent after only twenty-five minutes of carbon monoxide.

No glycogen determinations were made on the livers of these animals, but it is evident, from the initial hyperglycemia following carbon monoxide, that an ample amount of this material was present, and the

animals were fed until the second experiment, on the same carbohydrate-rich diet.

These results are also shown in curve form in figure 5 and they indicate that insulin prevents, or at least greatly depresses, the hyperglycemia which follows the administration of carbon monoxide to well-fed rabbits.

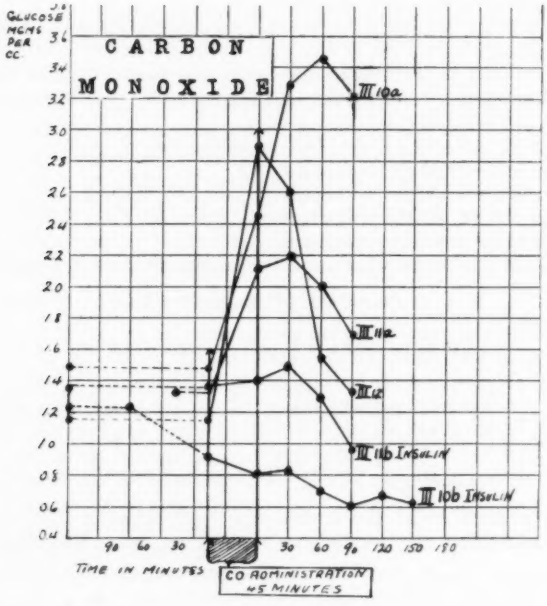

Fig. 5

Ether hyperglycemia. It is well known that the percentage of blood sugar rises during ether anesthesia. In rabbits the degree of this hyperglycemia, according to Fujii, is proportional to the intensity of the etherization. Of course it is impossible to be certain that the animals used in a series of experiments such as this, are all etherized to the same degree, but nevertheless, even under very light anesthesia, a certain degree of hyperglycemia always develops, provided of course the liver contains an ample supply of glycogen. The following experiments illustrate the changes in blood sugar following ether administrations to normal well-fed rabbits.

1. Rabbit IV, 7; weight, 1.75 kilos

Normal blood at 12:00—0.147 per cent sugar.
Ether commenced at 12:15.
Blood at 12:50—0.45 per cent sugar.
Blood at 1:25—0.50 per cent sugar.

2. Rabbit II, 14a

No normal sample of blood was examined.
Ether commenced at 10:15, continuous to 1:15.
Blood at 10:30—0.220 per cent sugar.
Blood at 11:30—0.355 per cent sugar.
Blood at 12:30—0.381 per cent sugar.
Blood at 1:15—0.459 per cent sugar, obtained from the heart.
Animal died 1.15.

3. Rabbit II, 14b

No normal sample of blood was examined.
Ether commenced at 10:35, continuous to 5:00.
Blood at 10:50—0.201 per cent sugar.
Blood at 11:50—0.365 per cent sugar.
Blood at 12:50—0.415 per cent sugar.
Blood at 1:50—0.447 per cent sugar.
Blood at 2:50—0.435 per cent sugar.
Blood at 3:50—0.440 per cent sugar.
Blood at 4:50—0.461 per cent sugar.
Animal died at 5:00.

From these results, which are also plotted in curves, figure 5, we see that the rise in blood sugar is rapid, and that it persists as long as the etherization: in each case until the death of the animal. Turning now to the experiments, in which ether was administered after injecting the animal with insulin, the following results were obtained:

Rabbit IV, 10; weight, 2.7 kilos; sugar-fed

Normal blood at 9:50—0.095 per cent sugar.
At 10:00, 7.0 cc. of insulin, subcutaneously.
Blood at 10:45—0.073 per cent sugar.
At 11:45, 5.0 cc. of insulin, subcutaneously.
Blood at 1:25—0.057 per cent sugar.
Blood at 2:30—0.052 per cent sugar.
At 2:35, 5.0 cc. of insulin, subcutaneously.
At 2:40 ether commenced, continuous to 4:25.
Blood at 3:10—0.065 per cent sugar.
Blood at 3:40—0.060 per cent sugar.
Blood at 4:10—0.071 per cent sugar.
Animal died at 4:25. Liver contained 3.91 per cent glycogen.

Rabbit IV, 8; weight 2.5 kilos

Normal blood at 11:00—0.127 per cent sugar.
At 11:15, 10.0 cc. of insulin, subcutaneously.
Blood at 2:00—0.037 per cent sugar.
Ether commenced at 3:00, continuous to 6:00.
At 3:01, 10.0 cc. of insulin, subcutaneously.
Blood at 3:45—0.095 per cent sugar.
Blood at 4:30—0.081 per cent sugar.
Blood at 5:30—0.045 per cent sugar.
Blood at 6:05—0.035 per cent sugar obtained from heart.
Animal killed 6:00. Liver contained 2.8 per cent glycogen.

These results are also given in curve form, figure 6, and they show that even with considerable amounts of glycogen in the liver there was only a slight and transient increase in the percentage of blood sugar while the animal was under ether.

In another case, insulin was not given until some time after the animal had been lightly under ether, with the following results:

Rabbit IV, 6; weight, 1.85 kilos; sugar-fed

Normal blood at 1:30—0.114 per cent sugar.
Ether commenced at 1:45 p.m., continuous to 5:15 p.m.
Blood at 2:15—0.142 per cent sugar.
Blood at 2:45—0.150 per cent sugar.
At 2:50, 10.0 cc. of insulin, subcutaneously.
Blood at 3:20—0.120 per cent sugar.
Blood at 3:50—0.082 per cent sugar.
Blood at 4:40—0.060 per cent sugar.
Blood at 5:10—0.047 per cent sugar.
Animal killed at 5:15. Liver contained 0.77 per cent glycogen.

The degree of hyperglycemia in this experiment is somewhat less striking possibly because of the relatively low glycogen content of the liver. The effect of insulin is however quite definite.

The effect of insulin in inhibiting the hyperglycemia of ether anesthesia is of importance from both experimental and clinical standpoints. From the former because it offers greater opportunities for the experimental investigation of the exact mechanism of the physiological action of insulin and from the latter, because it offers a means by which hyperglycemia may be controlled when surgical anesthesia is necessary in diabetic patients.

Discussion. Considering these results as a whole there can be no doubt that insulin in suitable dosage more or less inhibits the development of hyperglycemia in rabbits subjected to various conditions which

otherwise cause it. The degree of this inhibition is usually sufficient entirely to mask any rise in blood sugar but sometimes a rise occurs to a certain extent. This rise may be quite marked when the insulin is

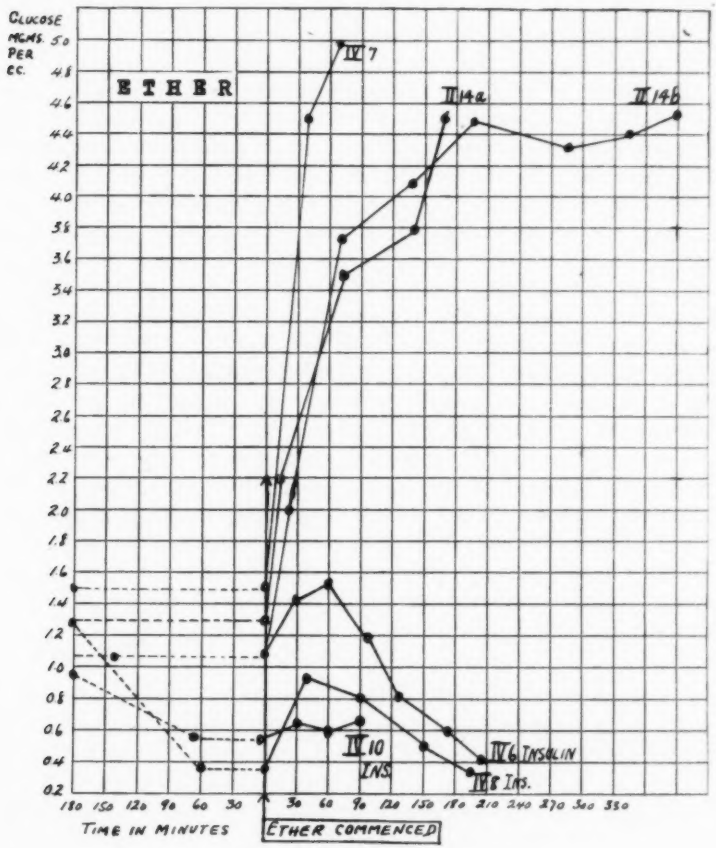

Fig. 6

injected at the same time as the application of the stimulus used to cause hyperglycemia, as is especially evident when this is epinephrin. In the latter case, indeed, the degree of hyperglycemia may be as great as the usual when the injections of epinephrin and insulin are made simultaneously. This would seem to indicate that there is a considerable

latent period before insulin unfolds its full action, a conclusion which is borne out by the preliminary observations which we have made on the behavior of the respiratory quotient following insulin. During this preliminary stage a powerful hyperglycemia-producing stimulus like epinephrin can apparently almost annul the effect of insulin. Further work on this aspect of the problem is in progress.

That insulin acts not only in the experimental forms of hyperglycemia discussed in this paper but also in that following pancreatectomy and in diabetes in man indicates that its action on carbohydrate metabolism is fundamental. Its effect on blood sugar is just as striking in a pancreatic diabetic animal with only a trace of glycogen in the liver as on a normal one whose liver is loaded with this material. In the former case insulin also influences the excretion of ketone bodies, the mobilization of fat and the respiratory quotient, which must be interpreted as meaning that it is essential in the regulation of the series of intermediary metabolic changes that culminate in the complete utilization of both fat and carbohydrate. By more intensive investigations of the metabolism of pancreatic diabetic animals treated with insulin it is therefore to be hoped that some light may be thrown on the problem of ketogenesis.

Concerning the modus operandi of insulin in preventing the purely experimental form of hyperglycemia, we have no hypothesis to offer. Before any such is attempted it will be necessary to obtain precise data on the amounts of glycogen in the liver and muscles before and during and after insulin action. Only then can it be known whether insulin actually stimulates glycogenesis to the extent that it takes glucose away from the systemic blood.

CONCLUSIONS

1. When the fall in blood sugar due to subcutaneous injection of insulin is thoroughly established, piqûre, epinephrin, mechanical and carbon monoxide asphyxia and ether do not cause the usual degree of hyperglycemia. There may be a distinct increase in the percentage of blood sugar but very seldom is this sufficient to raise it to the normal level existing before insulin was given.

2. Even when the insulin is given at the same time as the animal is subjected to the experimental condition used to cause hyperglycemia, the latter may be either entirely absent or greatly diminished.

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